Arbuscular mycorrhiza: the mother of plant root endosymbioses

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Abstract | Arbuscular mycorrhiza (AM), a symbiosis between plants and members of an ancient phylum of fungi, the Glomeromycota, improves the supply of water and nutrients, such as phosphate and nitrogen, to the host plant. In return, up to 20% of plant-fixed carbon is transferred to the fungus. Nutrient transport occurs through symbiotic structures inside plant root cells known as arbuscules. AM development is accompanied by an exchange of signalling molecules between the symbionts. A novel class of plant hormones known as strigolactones are exuded by the plant roots. On the one hand, strigolactones stimulate fungal metabolism and branching. On the other hand, they also trigger seed germination of parasitic plants. Fungi release signalling molecules, in the form of 'Myc factors' that trigger symbiotic root responses. Plant genes required for AM development have been characterized. During evolution, the genetic programme for AM has been recruited for other plant root symbioses: functional adaptation of a plant receptor kinase that is essential for AM symbiosis paved the way for nitrogen-fixing bacteria to form intracellular symbioses with plant cells.

Aseptate
Not containing septae.

Coenocytic
Multiple nuclei within the same cell.

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doi:10.1038/nrmicro1987

Plant root symbioses with fungi occur in several different forms and are referred to as mycorrhiza (from the Greek 'mycos', meaning fungus and 'rhiza', meaning root). In ectomycorrhiza, which is predominant on trees in temperate forests, the fungal partner remains outside of plant cells, whereas in endomycorrhiza, including orchid, ericoid and arbuscular mycorrhiza (AM), part of the fungal hyphae is inside. AM is probably the most widespread terrestrial symbiosis[1] and is formed by 70–90% of land plant species[2] with fungi that belong to a monophyletic phylum, the Glomeromycota[3,4] (FIG. 1). Symbiotic development results in the formation of tree-shaped subcellular structures within plant cells. These structures, which are known as arbuscules (from the Latin 'arbusculum', meaning bush or little tree) are thought to be the main site of nutrient exchange between the fungal and plant symbiotic partners (FIG. 2). AM intimately connects plants to the hyphal network of the fungi, which can be in excess of 100 metres of hyphae per cubic centimetre of soil[5]. This hyphal network is specialized for nutrient (predominantly phosphate) and water uptake[6]. In return for supplying plants with nutrients and water, AM fungi obtain carbohydrates from plants[7,8]. Up to 20% of the photosynthesis products of terrestrial plants (approximately 5 billion tonnes of carbon per year) are estimated to be consumed by AM fungi[9]. Therefore, AM symbiosis contributes significantly to global phosphate and carbon cycling and influences primary productivity in terrestrial ecosystems[1]. The beneficial effects of AM are most apparent under conditions of limited nutrient availability. Although the underlying regulatory mechanisms are not understood, the amount of root colonization typically decreases when nutrients are in abundance. Interestingly, the colonization of roots with AM fungi has been observed to lead to an inhibition of bacterial leaf pathogens[10]. Whether such increased resilience to pathogens is a consequence of improved plant fitness or is due to specific defence responses that are induced by AM fungi is unknown.

AM fungi are unusual organisms because of their age, lifestyle and genetic make-up; they have existed for more than 400 million years morphologically unaltered and could therefore qualify as living fossils. They are considered by many to be ancient asexuals, a characteristic that defies the predictions of evolutionary theory. The hyphal network of AM fungi is usually aseptate and coenocytic, with hundreds of nuclei sharing the same cytoplasm. Likewise, individual spores contain hundreds of nuclei and the question of how the different polymorphic DNA-sequence variants that are present

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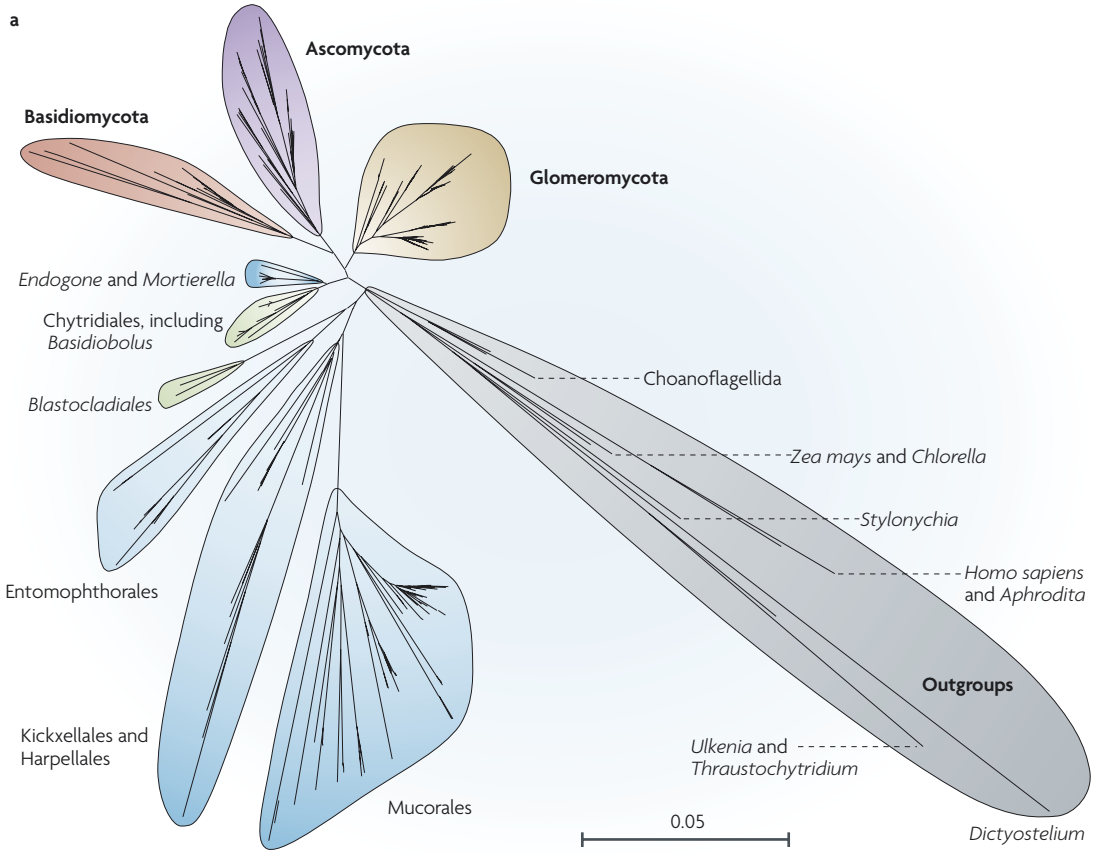

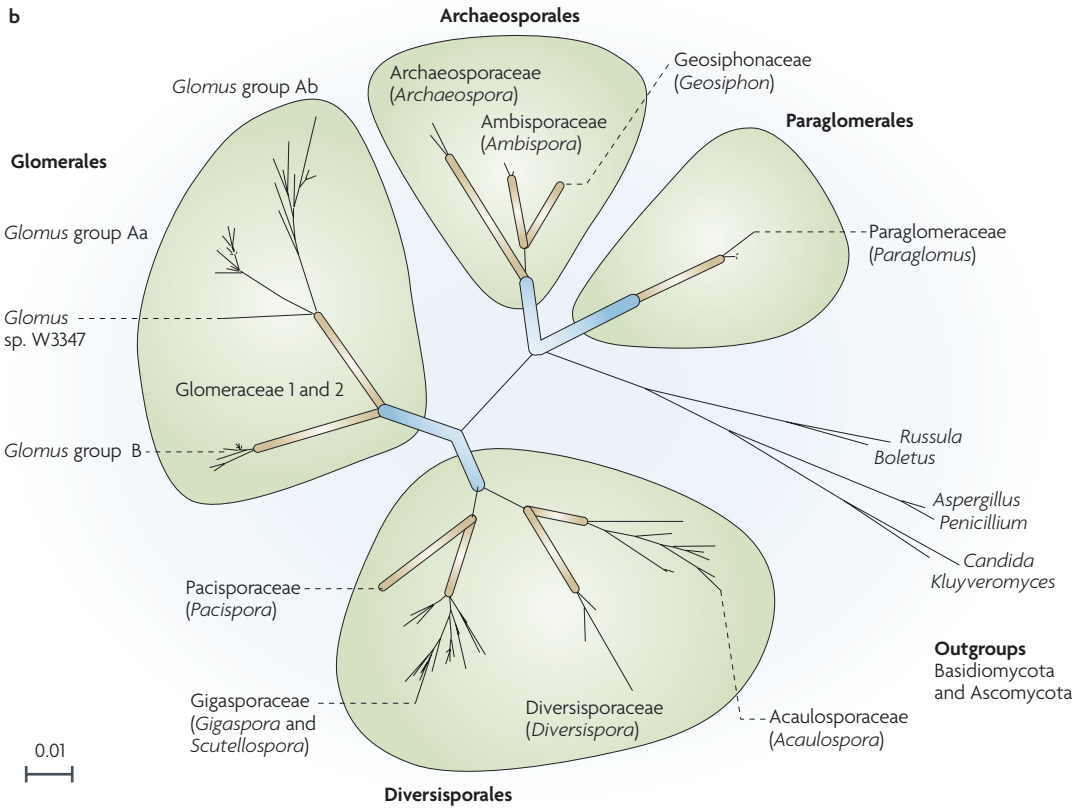

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Anastomosis
A hyphal fusion with a cytoplasmic connection.

Obligate biotroph
An organism that is unable to complete a reproductive cycle in the absence of a living host.

Mycoheterotrophic
Obtains carbon sources from a fungal symbiont.

within a single cell are distributed between genomes or nuclei is the subject of an ongoing debate[11–13]. Although there is no confirmed report of a sexual stage in the life cycle of AM fungi, it is possible that genetic material is exchanged and recombined; anastomosis between hyphae[14,15] allows the exchange of nuclei[15] but has so far only been observed between hyphae of closely related fungal strains. It will be interesting to determine the level of relatedness that is required for these fusions to occur. Molecular evidence for recombination in AM fungi[16,17] has been controversial[13]. As an important step towards the genetic manipulation of these fungi, transient transformation by particle bombardment has been achieved[18].

Although spores of AM fungi can germinate in the absence of host plants, they are obligate biotrophs, and therefore depend on a living photoautotrophic partner to complete their life cycle and produce the next generation of spores. In one reported case, however, co-culture with *Paenibacillus validus* led to the production of secondary and infective spores in the absence of a host[19].

Individual fungal strains exhibit little host specificity when grown with different plants under laboratory conditions[2]. Likewise, a single plant can be colonized by many different AM fungal species within the same root[1,20]. Therefore, on the one hand, the AM symbiosis is thought to show little host specificity at the level of colonization. On the other hand, the biodiversity of fungal and plant communities are positively correlated with each other[21] and host preference seems to play an important role in natural ecosystems[22,23]. It is likely that these host preferences reflect different fungal strategies and levels of functional compatibility[24,25] (FIG. 3). High specificity has been observed between mycoheterotrophic plants and their mycosymbionts[26]. Most AM fungi that can be detected in natural ecosystems have not been cultured[1] and it is possible that many have a more restricted host range than 'generalists', such as *Glomus mossae* or *Glomus intraradices*, which are intensively investigated because they are easily cultured.

This Review will describe AM development, an area in which substantial progress has been made over the past few years. Two novel signalling molecules have been identified that alter fungal development or plant gene expression. Seven plant genes that are involved in symbiotic reprogramming of plant cell development have been cloned. This reprogramming involves the formation of a newly discovered prepenetration apparatus (PPA) by the plant cell in anticipation of fungal infection, by which the plant cell dictates the route of fungal intracellular passage. Moreover, our current knowledge of the function of the AM symbiosis is summarized, including the nutrient exchange and metabolite fluxes in AM. Finally, evolutionary aspects of AM fungi and the evolution of the plant genetic programme for symbiosis development are considered.

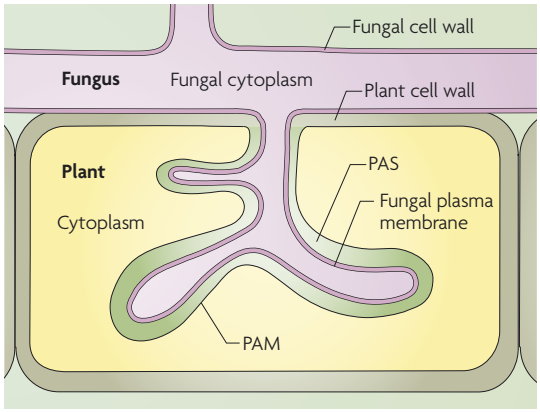

Figure 2 | **The arbuscule.** Schematic drawing of an arbuscule, the symbiotic structure and arbuscular mycorrhiza (AM). Each fungal branch within a plant cell is surrounded by a plant-derived periarbuscular membrane (PAM) that is continuous with the plant plasma membrane and excludes the fungus from the plant cytoplasm. The apoplastic interface between the fungal plasma membrane and the plant-derived PAM is called the periarbuscular space (PAS). Because of the cell-wall synthesizing potential of both the fungal membrane and the PAM, the PAS comprises fungal and plant cell-wall material.

AM development

The presymbiotic phase. Multiple, successive rounds of spore germination and retraction of nuclei and cytoplasm can occur in AM fungi. This exploratory hyphal development changes dramatically in the presence of plant-derived signals (FIG. 4). The stimulatory effect of plant root exudates on AM fungal hyphae has been recognized for a long time, but the molecular identity of the 'branching factors' has only recently been identified. In two landmark papers, strigolactones were found to be responsible for the induction of branching[27] and alterations in fungal physiology and mitochondrial activity[28]. Strigolactones can also stimulate spore germination in some AM fungi. Strigolactones are short-lived in the rhizosphere owing to a labile ether bond that spontaneously hydrolyses in water. This ephemeral compound forms a steep concentration gradient, and therefore its perception has been suggested to be a reliable indicator of the proximity of a host root[29]. Interestingly, the same class of compounds was identified 50 years ago as a potent germination inducer of seeds of the parasitic plant genus *Striga*. The discovery that strigolactones

◀ Figure 1 | **Arbuscular mycorrhiza (AM) fungi form an independent phylum, the Glomeromycota. a** | A phylogenetic tree showing the Glomeromycota in relation to other main fungal lineages: the Ascomycota and Basidiomycota and the non-monophyletic Chytridiomycota (green) and Zygomycota (blue)[3]. All tested members of the Glomeromycota form AM and all AM fungi are members of the Glomeromycota. **b** | Phylogenetic relationships between members of the Glomeromycota. Among the four orders that are currently recognized, the Archaeosporales and Paraglomerales are clearly distinct from the subgroup Glomerales and Diversisporales. The phylogeny and taxonomy of AM fungi is still under substantial debate. For example, owing to the significant divergence among the Glomeraceae, this family will probably be taxonomically separated in the future. The scale bar represents the number of substitutions per site. Panel **a** modified, with permission, from REF. 3 (2001) Cambridge University Press. Panel **b** modified, with permission, from REF. 122 (2002) Springer Netherlands.

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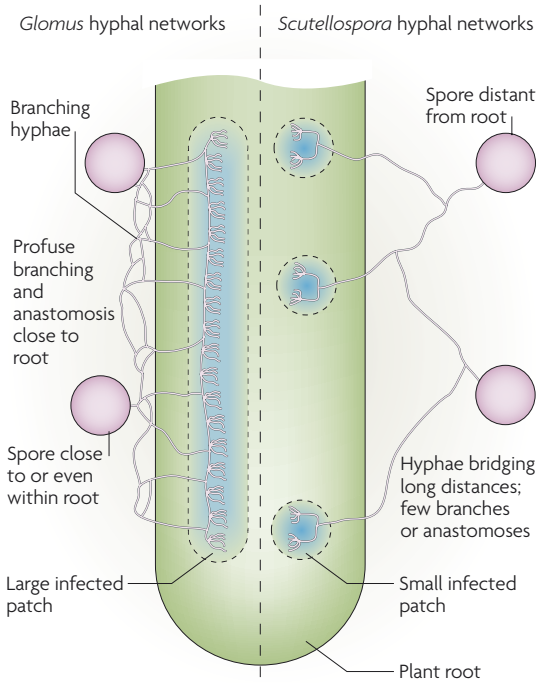

Figure 3 | **Different hyphal growth and branching strategies in arbuscular mycorrhiza (AM) fungi.** AM fungi have different hyphal growth patterns, anastomoses and branching frequencies. These differences probably reflect different strategies and the occupation of different niches within the soil. Many *Glomus* species form highly branched and anastomosing hyphal networks. These networks are more recalcitrant to disturbances of the soil than the mycelia of species of *Scutellospora* or *Gigaspora*, which form longer hyphae and can probably explore more distant regions of the soil[14,123]. Most of the fungal biomass in members of the Gigasporaceae family is found in the hyphae that are located outside the plant root, whereas in members of the Glomeraceae family, most of the hyphal biomass is inside the root[21].

act as signals for AM fungi has revealed that species of *Striga* exploit a conserved and ancient communication system between symbiotic fungi and their host plants[29]. The recent pioneering discovery of strigolactones as novel endogenous plant hormones in diverse angiosperms that range from *Arabidopsis thaliana* to pea and rice[30,31] suggests that the strigolactone perception system of *Striga* is less unique than previously thought. By contrast, it is probably derived from a general hormonal perception system of angiosperms. Whether strigolactones first evolved as endogenous plant hormones or as signals in AM remains an open question. Strigolactone perception by the fungus induces the so-called presymbiotic stage, which is characterized by continued hyphal growth, increased physiological activity and profuse branching of hyphae. Plant mutants that are unable to produce strigolactones are now available in pea and rice[30], and can be useful for refined functional analyses of these signalling molecules in AM development.

Nod factors
The bacterial symbionts of legumes (rhizobia) produce signalling molecules named Nod factors. They consist of an *N*-acetylglucosamine backbone that carries various strain-specific decorations including a lipid side chain.

Calcium spiking
A sharp periodic increase in calcium concentration around the nucleus of symbiotically stimulated root cells.

Fungal signalling molecules and plant receptors. There is currently much interest in the molecular identification of fungal signalling molecules that induce symbiosis-specific responses in the host root (collectively called Myc factors). The existence of such molecules became apparent in experiments in which direct contact between the fungus and plant was prevented by fungus-impenetrable membranes. In these experiments, a symbiosis-responsive *ENOD11*-promoter GUS (β-glucuronidase) reporter gene fusion in roots of *Medicago truncatula* was activated in the vicinity of fungal hyphae[32]. This Myc factor was found to be a diffusible molecule that induced transcriptional activation of symbiosis-related genes. Whether the production of this Myc factor is stimulated by strigolactones is unclear. A weak but significant increase in lateral root initiation was observed when roots were treated with a diffusible factor from AM fungi[33]. However, it is unknown whether the root-inducing and *ENOD11*-inducing molecules are the same. Calcium signatures that were reminiscent of, but clearly distinct from, Nod-factor-induced calcium spiking were recently observed in root hair cells in the vicinity of, but before contact with, approaching AM hyphae[34]. Interestingly, calcium oscillations of lower frequency and amplitude than the Nod-factor-induced calcium spiking can be induced by oligo-*N*-acetylglucosamine[35,36]. LysM domains have been implicated in *N*-acetylglucosamine binding. Two different receptor-like molecules that are required for chitin perception, both of which have LysM domains in their extracellular domain, have recently been identified in rice and *A. thaliana*[37,38]. These receptor molecules are involved in the induction of resistance responses and constitute part of an ancient perception system for the detection of microbial-associated molecular patterns (MAMPs). The Nod factor receptors also contain LysM domains, which are likely to bind the lipochitooligosaccharide Nod factors[39,40]. Their close structural relationship indicates that the Nod factor and chitin receptors share a common ancestry. AM fungi have chitin in their cell walls and could be recognized by the chitin-perception system. In addition to chitin and Nod factor receptors, both *A. thaliana* and rice contain several LysM-containing receptor kinases of unknown function. Whether, in common with rhizobia, AM fungi produce chitooligosaccharides or derivatives that function as symbiotic signals which are recognized by LysM receptor kinases remains to be determined. The search for the Myc factor requires a specific assay system, because plant cells are responsive to a range of MAMPs[41] that trigger related downstream responses, including calcium responses[42]. Therefore, the challenge is to show that candidate Myc factors, such as an as-yet-unidentified small molecule from *Gigaspora margarita*, which elicits calcium responses in soybean cells, can induce symbiosis-specific responses[43].

The prepenetration apparatus. The paradigm-shifting discovery that the plant cell actively prepares the intracellular environment for AM fungal hyphae[44,45] changed our view of the role of the plant cell during

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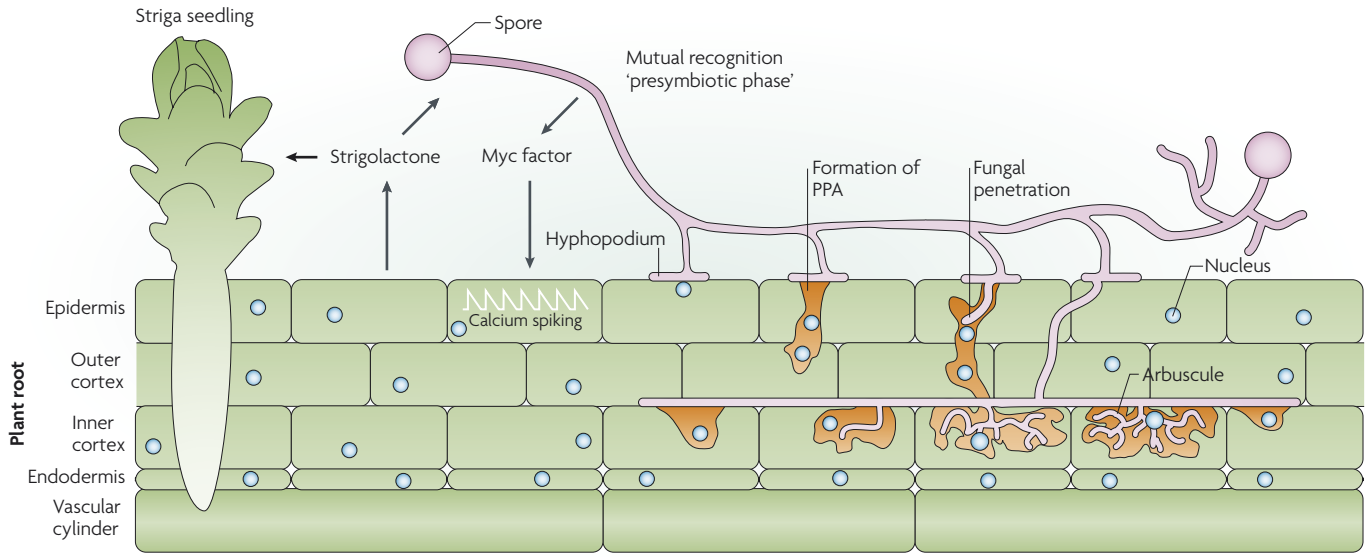

Figure 4 | **Steps in arbuscular mycorrhiza (AM) development.** Plant roots exude strigolactones which induce spore germination and hyphal branching and increase physiological activity in fungal spores and hyphae. Strigolactones also induce seed germination in parasitic plants, such as *Striga*[124]. Fungi produce mycorrhiza (Myc) factors that are operationally defined through their ability to induce calcium oscillations in root epidermal cells[34] and to activate plant symbiosis-related genes[32]. AM fungi form special types of appressoria called hyphopodia, which by definition develop from mature hyphae and not from germination tubes[125]. As a consequence of sequential chemical and mechanical stimulation, plant cells produce a prepenetration apparatus (PPA). Subsequently, a fungal hypha that extends from the hyphopodium enters the PPA, which guides the fungus through root cells towards the cortex. Here, the fungus leaves the plant cell and enters the apoplast, where it branches and grows laterally along the root axis. These hyphae induce the development of PPA-like structures in inner cortical cells[45], subsequently enter these cells and branch to form arbuscules. Vesicles, which are proposed to function as storage organs of the fungus, are sometimes, but not always, formed in AM and are present in the apoplast (not shown). New spores are typically synthesized outside of the plant root at the leading tip of individual fungal hyphae. Figure modified, with permission, from REF. 45 (2008) © American Society of Plant Biologists.

infection by biotrophic fungi. The PPA is a subcellular structure that predetermines the path of fungal growth through the plant cell and is formed 4–5 hours after the formation of a fungal appressorium, also called a hyphopodium. Formation of the PPA is preceded by a migration of the plant cell nucleus towards the point of anticipated fungal entry. The nucleus then moves ahead of the developing PPA, as if to guide its growth direction through the cell. The PPA is a thick cytoplasmic bridge across the vacuole of the host cell. It contains cytoskeletal microtubules and microfilaments, which together with dense endoplasmic reticulum cisternae form a hollow tube within the PPA that connects the leading nucleus with the site of appressorial contact[44,46] (FIG. 5). Only after this 'transcellular tunnel' is completed can the fungal hypha penetrate the host cell. Endoplasmic reticulum membranes that decorate the tunnel are ideally positioned for the synthesis of the perifungal membrane. However, the signals that trigger the formation of the PPA[44] are unknown. Purely mechanical stimulation of plant cells with a needle can induce the nucleus to migrate towards the site of disturbance[47]. This might be the initial trigger during AM, as this response is independent of the common plant *SYM* genes *DMI2* and *DMI3*. To induce formation of the PPA, however, additional chemical cues are probably needed to provide specificity. The structurally related 'pre-infection thread' of legumes, which forms in response to rhizobia[48] in anticipation of bacterial infection, probably evolved from the PPA (FIG. 5).

Plant genes required for AM development

At least seven genes that are required for both the AM symbiosis and the root-nodule symbiosis with rhizobia have been identified in legumes[49] (TABLE 1). These genes encode proteins that are directly or indirectly involved in a signal transduction network that is required for the development of intracellular accommodation structures for symbiotic fungi and bacteria by the host cell (FIGS 5,6). The AM phenotype of a mutant that is defective in a common symbiosis gene is characterized by an early block of fungal infection in the outer cell layers[49]. Phenotypic analysis of *M. truncatula* symbiotic mutants shows that the common *SYM* genes *DMI2* and *DMI3* (TABLE 1) are required for PPA induction[44] and that *DMI3* is required for a subset of genes to be induced during PPA formation[46]. Transcriptome analysis revealed that most AM-induced genes are not activated in common *sym* mutants[46,49,50]. Similarly, the transcriptional response to Nod factors is largely abolished[51].

An analysis of calcium spiking in *L. japonicus* in response to Nod factor revealed that *symrk, castor, pollux, nup85* and *nup133* mutants are defective for

Appressorium
A flattened, hyphal organ that facilitates the penetration of cells or tissues of other organisms.

Microfilament
Strong, but flexible, linear polymer of actin subunits and component of the cytoskeleton.

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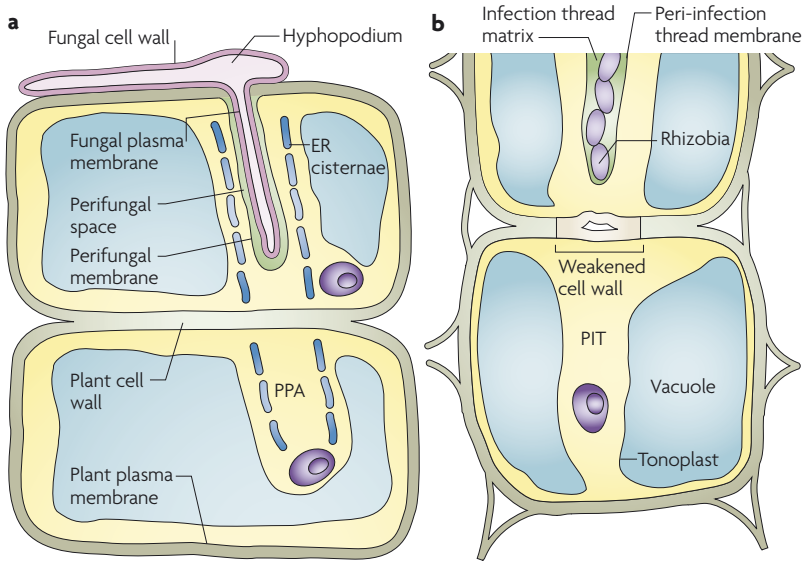

Figure 5 | **Comparison of intracellular accommodation structures in bacterial and fungal root endosymbioses. a** | The prepenetration apparatus (PPA) is a cytoplasmic bridge across the vacuole of a plant cell that forms in anticipation of fungal infection (lower cell). The plant cell nucleus migrates ahead of the growing PPA and determines its orientation within the cell. The PPA contains a hollow tube that is formed by microtubules and is lined with endoplasmic reticulum cisternae. Only after completion of the PPA does a fungal hypha enter the PPA (upper cell). **b** | A preinfection thread (PIT) forms ahead of the bacteria-filled infection thread. The PIT can be induced by bacterial signals alone[48] and contains an array of microtubules that resemble the arrangement within the PPA[54]. The PIT is unique to the nodulating clade and is likely to have evolved from the PPA of arbuscular mycorrhiza (AM). A plant-derived perimicrobial membrane encloses the bacteria-filled infection thread and the fungal hypha and prevents microbial contact with the plant cytoplasm. This membrane synthesises cell wall material, which contributes to the composition of the apoplastic interface between the symbiotic organisms. Part **a** modified, with permission, from REF. 44 (2005) American Society of Plant Biologists and REF. 45 (2008) American Society of Plant Biologists. Part **b** modified, with permission, from REF. 118 (2000) Elsevier Science.

calcium spiking, whereas *CCaMK* and *CYCLOPS* act downstream[52,53]. The data obtained for the AM-induced calcium signatures in *M. truncatula* mutants are consistent with those for Nod factor, in that *dmi1* and *dmi2* do not show this response, whereas *dmi3* mutants do[34]. Mutants that have defective common *SYM* genes do not form infection threads and, with the exception of *cyclops* mutants, do not initiate nodule organogenesis[55,56]. This suggests that the common *SYM* gene products are involved in the early stages of symbiotic signal transduction, which involves the generation and decoding of calcium oscillations in and around the nucleus and causes the induction of early symbiosis-related gene expression. Consistent with this, some of the predicted protein products are typical signal transduction components, although the contribution of the nucleoporins is likely to be indirect (FIG. 6; TABLE 1). There are subtle differences in the AM phenotypes of common *sym* mutants in the epidermis, the outer cortical cell layers and the arbuscule-forming cells[57] (TABLE 1). For example, there is a clear requirement for *CCaMK* and *CYCLOPS* in arbuscule development, whereas arbuscules can develop normally in *symrk* mutant roots. This is indicative of substantial plasticity, and probably cell-type-specific functionality, in the signalling network that is defined by the common SYM proteins. Moreover, accumulating evidence suggests there is common *SYM*-independent signalling[32,46]. TABLE 1 and FIGURE 6 provide an overview over the common SYM pathway, whereas the following sections describe individual components.

SYMRK. *SYMRK* (also known as *M. truncatula DMI2* or *Medicago sativa NORK*) encodes a receptor-like kinase[58,59] that has an enzymatically functional kinase domain[60]. Owing to the structure of SYMRK and the symbiotic phenotype of corresponding mutants, this molecule is typically portrayed as the entry point into the common symbiotic signalling pathway. In this model, SYMRK has the potential to directly or indirectly perceive extracellular signals from microbial symbionts and transduce this perception event through its intracellular kinase domain. The ligands of the SYMRK extracellular domain have not been identified, however. Interestingly, there are at least three different types of SYMRK in the angiosperm lineage which differ in length and the domain structure of their predicted extracellular regions. The shortest type (found in rice) is sufficient for restoring AM in *Lotus symrk* mutants, whereas the full-length extracellular extension of SYMRK only seems to be required during interactions with rhizobia[61]. It is therefore possible that during AM and root-nodule symbiosis different extracellular ligands bind to different parts of the SYMRK extracellular domain. SYMRK can be exchanged between *M. truncatula* and *Lotus japonicus*, and the corresponding complemented *Lotus* and *Medicago symrk* mutant roots regain their ability to form nodules with *Mesorhizobium loti* and *Sinorhizobium meliloti*, respectively. This indicates that SYMRK is not involved in determining rhizobial recognition specificity. Even SYMRK from actinorhiza plants that nodulate with Gram-positive bacteria of the genus *Frankia* or non-nodulating eurosid species restores nodulation and AM in *Lotus symrk* mutants[61,62]. Therefore, SYMRK does not contribute to recognitional specificity, and probably does not directly bind to the Nod factor[61].

CASTOR *and* POLLUX. Mutants that are defective in *CASTOR* or *POLLUX* (also known as pea *SYM8* and *Medicago DMI1*)[63] are also defective in Nod-factor-induced calcium spiking[64,65]. CASTOR and POLLUX (or DMI1) share similar overall domain structures and high sequence similarity[65]. Electrophysiological measurements of channels that were reconstituted in lipid membranes and yeast-complementation experiments unambiguously showed that these proteins are potassium-permeable cation channels (M. Charpentier and colleagues, personal communication). Importantly, these proteins are much less permeable for calcium, which indicates that they are unlikely to be the channels that release calcium from the storage compartment. Nuclear localization of the CASTOR and POLLUX proteins is consistent with their proposed role as counter-ion channels that compensate for the rapid charge imbalance that is produced during calcium spiking (M. Charpentier and colleagues, personal communication).

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Table 1 | Overview of common *sym* mutants and their corresponding phenotypes

Gene	Mutants			Phenotypes of *Lotus* mutants			Predicted function of gene product
	Lotus japonicus (previous designation)	*Medicago truncatula*	*Pisum sativum*	Arbuscular mycorrhiza (AM) phenotype*	Root-nodule symbiosis phenotype‡	Calcium spiking§	
SYMRK	*symrk*[59] (*sym2*)	*dmi2* (REF. 58) and *Medicago sativa nork*	*sym19* (REF. 126)	Type II	Non-nodulating‖	No	Leucine-rich-repeat receptor kinase[60]
CASTOR	*castor*[65] (*sym4* and *sym71*)	Unknown	Unknown	Types II and III	Non-nodulating‖	No	Cation channel
POLLUX	*pollux*[65] (*sym23* and *sym86*)	*dmi1* (REF. 127)	*sym8* (REF. 127)	Type II	Non-nodulating‖	No	Cation channel
NUP85	*nup85* (REF. 67) (*sym24*, *sym73* and *sym85*)	Unknown	Unknown	Type II temperature sensitive	Temperature sensitive¶	No	Putative nuclear pore component
NUP133	*nup133* (REF. 66) (*sym3* and *sym45*)	Unknown	Unknown	Type II temperature sensitive	Temperature sensitive#	No	Putative nuclear pore component
CCaMK	*ccamk*[73] (sym15 and sym72)	*dmi3* (REFS 70,71)	*sym9* (REF. 128)	Types I, II and III	Non-nodulating‖**	Yes	Calcium and calmodulin-dependent protein kinase
CYCLOPS	*cyclops* (*sym6*, *sym30* and *sym82*)‡‡	*ipd3* (REF. 129)§§	Unknown	Types II and III	Small, non-infected nodules‖‖	Yes	Unknown protein that features a nuclear localization signal and a carboxy-terminal coiled-coil domain

*Arbuscular mycorrhiza (AM) common *sym* mutant phenotypes I–III are characterized by impaired epidermal opening (type I), impaired intracellular passage through the outer cell layer (or layers) (type II) and/or impaired arbuscule formation (type III). ‡Based on phenotypes described in REFS 115,130–132. §Based on phenotypes described in REFS 52,53. ‖Root-hair swelling and branching occurs after inoculation with *Mesorhizobium loti*, but neither infection threads nor nitrogen-fixing nodules are formed. ¶More arbuscules and nodules form at 18°C compared with 22°C[67]. #Few nodules form at 22°C, and almost no nodules form at 26°C[66]. **Deregulated versions of CCaMK induce spontaneous nodule formation in the absence of rhizobia[72,73]. ‡‡K. Yano and colleagues, personal communication. §§Mutants not available. ‖‖*cyclops* mutants form non-fixing, small white bumps after inoculation with *M. loti* (K. Yano and colleagues, personal communication).

Nucleoporins. Two genes that encode proteins with similarity to nucleoporins 85 and 133 are required for the temperature-dependent initiation of symbiosis[66,67] (FIG. 6; TABLE 1). In humans and yeast, both of these proteins belong to the same NUP107–160 subcomplex of the nuclear pore and are not in contact with substrates of the canonical import and export pathways[68]. However, the transport of proteins that are larger than 75 kDa to the inner nuclear envelope is not well understood[69]. Because *Lotus* NUP85 and NUP133 act upstream of calcium spiking, a plant version of the vertebrate NUP107–160 complex might be involved in transporting CASTOR or POLLUX or both to the inner nuclear envelope. Considering the central role of the nuclear pore in transport processes into and out of the nucleus, it is surprising that *nup85* and *nup133* mutants lack major pleiotropic defects. This could be explained by either a partial redundancy of plant nuclear pore components or functional diversification of the symbiotic nucleoporin homologues.

CCaMK. A calcium–calmodulin-dependent protein kinase (CCaMK) is essential for AM[70,71]. The calmodulin-binding domain and calcium-binding EF hand motifs of CCaMK allow the protein to sense calcium, which makes it a prime candidate for the response to calcium signatures that are induced by AM fungi[34] or the Nod factor that induces calcium spiking. Interestingly, a deregulated version of the protein can trigger spontaneous nodule formation in the absence of rhizobia[72,73], which indicates that deregulation of CCaMK alone is sufficient to trigger the organogenesis programme. An unresolved conundrum is the observation that AM fungi induce calcium signatures but no nodules. Perhaps during AM, CCaMK is not activated to the same extent or in the same cell types as during nodulation. Alternatively, additional layers of negative regulation might be operating to inhibit nodule organogenesis during AM.

CYCLOPS. *cyclops* mutants severely impair the infection process of bacterial or fungal symbionts, and are also defective in arbuscule development[49]. During root-nodule symbiosis, *cyclops* mutants exhibit specific defects in infection-thread initiation, but not in nodule organogenesis (K. Yano and colleagues, personal communication), indicating that CYCLOPS acts in an infection-specific branch of the symbiotic signalling network. *CYCLOPS* encodes a protein with no overall sequence similarity to proteins with known function, but contains a functional nuclear localization signal and a carboxy-terminal coiled-coil domain. CYCLOPS interacts with CCaMK in yeast and *in planta* and can be phosphorylated by CCaMK *in vitro*.

The signalling network that enables symbiotic infection by AM fungi is starting to emerge from the analysis of these cloned plant genes. Additional insights are expected from the identification of the mutations in *Petunia* arbuscule-development mutants[74] and in maize AM mutants[74,75].

Arbuscule development

Arbuscules are the result of coordinated subcellular development of the host plant cell and the AM fungus.

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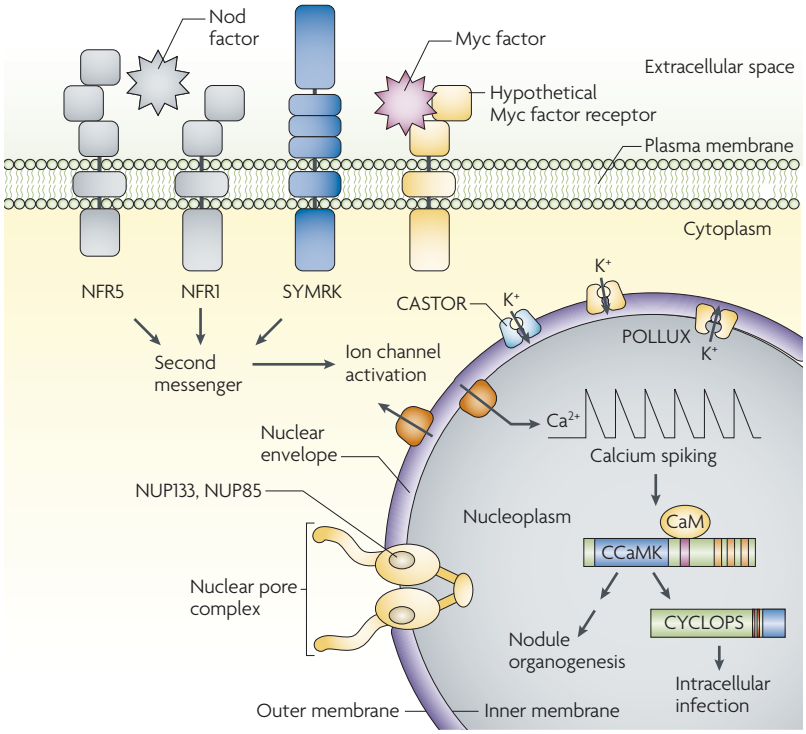

Figure 6 | **Common symbiosis signalling components for arbuscular mycorrhiza (AM) and root-nodule symbiosis.** Perception of AM fungal or rhizobia-derived signals triggers early signal transduction, which is mediated by at least seven shared components. The symbiosis receptor kinase SYMRK acts upstream of the Nod factor- and Myc factor-induced calcium signatures that occur in and around the nucleus[34]. Perinuclear calcium spiking involves the release of calcium from a storage compartment (probably the nuclear envelope) through as-yet-unidentified calcium channels. The potassium-permeable channels CASTOR and POLLUX might compensate for the resulting charge imbalance. The nucleoporins NUP85 and NUP133 are required for calcium spiking, although their mode of involvement is currently unknown. The calcium–calmodulin-dependent protein kinase (CCaMK) forms a complex with CYCLOPS, a phosphorylation substrate, within the nucleus. Together with calmodulin, this complex might decode the symbiotic calcium signatures (K. Yano and colleagues, personal communication). Upstream of the common pathway, the Nod factor receptor kinases NFR1 and NFR5 are specifically required for Nod factor perception[39]. It is possible that similar receptors are involved in Myc factor perception. *Lotus japonicus* protein nomenclature is used (see TABLE 1 for the names of common *SYM* gene orthologues of other species).

The fungal hyphae branch repeatedly to produce the tree-shaped arbuscule structure. The exact structure that is formed can vary depending on the fungal and host genotype[2]. The branches of the fungi are excluded from the host cytoplasm by a plant-derived periarbuscular membrane (PAM). Nutrients, and perhaps signals, are exchanged across the symbiotic interface between the fungus and the plant (which constitutes the PAM, the fungal plasma membrane and the periarbuscular space that exists between these two membranes (FIGS. 2,7))[76]. The transporters that mediate metabolite exchange at the interface between the plant and the fungus are of key biotechnological interest, and some candidate transporter genes have been cloned, although only the PT4 transporter has been specifically localized to the PAM[77]. The PAM is continuous with the plant plasma membrane, but has a distinct protein composition, as revealed by the observation that the *M. truncatula* phosphate transporter PT4 is present in the PAM but absent from the plasma membrane[77]. Ultrastructural analyses have detected molecules in the periarbuscular space that are typically found in the plant primary cell wall, including β-1,4-glucans, non-esterified homogalacturonans, xyloglucans, proteins that are rich in hydroxyproline and arabinogalactan proteins[78].

Arbuscules have a shorter lifetime than the host cell (perhaps as short as 8.5 days[79]), and consequently, a single host cell is thought to be competent for several rounds of successive fungal invasions. In a recent study, Javot and colleagues[80] analysed the time-scale of arbuscule development in more detail. They found that arbuscules undergo a phase of growth until a certain maximum size is reached, after which arbuscule degradation or senescence is induced and the arbuscular hyphae become separated from the remaining cytoplasm by septation. Arbuscules subsequently collapse over time and ultimately disappear. This succession of arbuscules is costly in terms of plant and fungal resources, so why are arbuscules so short-lived? The observation that mutation of the arbuscule-specific phosphate transporter PT4 results in premature degradation of arbuscules[80] suggests that the lifetime of arbuscules is influenced by their ability to deliver phosphate and probably other nutrients. This provides the plant with a means to maintain efficient arbuscules and penalize inefficient arbuscules with early degradation. Conceptually, this mechanism allows the plant not only to discriminate between efficient and inefficient fungal species but also to remove potientally 'good' fungal symbionts that are attached to a poor phosphate source. This concept allows fungal clones and species to compete for arbuscule formation, which allows succession in an established root system. The spatial distribution of nutrients in the soil will change over time, and well-connected hyphae replace 'non-providers'. Thus, a limited arbuscule lifetime allows constant renewal and rewiring of the hyphal network and allows connections to be made to the most efficient providers (FIG. 3). AM is a living fossil, and therefore mechanisms to specifically promote beneficial symbiotic fungi, and to counter-select against inefficient 'parasitic' fungi, might have contributed to the long-term evolutionary stability of this symbiosis. A special role in the development of arbuscules has been ascribed to lysophosphatidylcholine (LPC). LPC is a normal product of phospholipid metabolism and was recently described as a signalling molecule that activates the expression of phosphate-transporter genes, including the potato gene *PT3* (REF. 81). This type of phosphate transporter was found to be required for arbuscule maintenance[80]. A phosphate-containing molecule, such as LPC, might be a cell autonomous molecular measure for how much phosphate is made locally available to the plant.

Arbuscule development is accompanied by plastid proliferation and the formation of a plastidial network in close physical contact with the arbuscule[82]. The plastid is involved in numerous biosynthetic activities, including the production of apocarotenoids that specifically accumulate in AM roots[83]. Given the involvement of hormones in almost all plant developmental processes, it is thought that hormones have key roles during the development of

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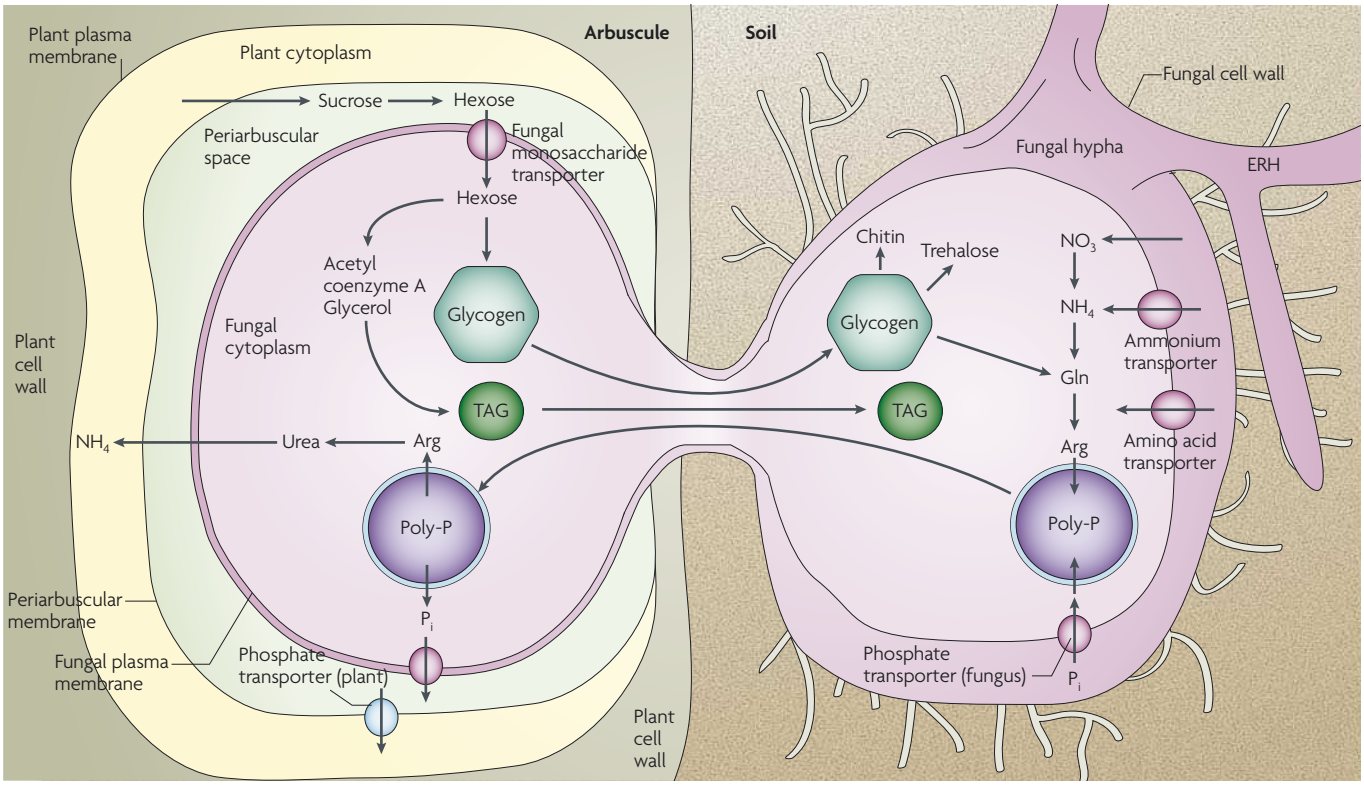

Figure 7 | **Metabolic fluxes and long-distance transport in arbuscular mycorrhiza (AM).** Plant-derived carbon is transported to the fungus through the two membranes at the symbiotic interface. This carbon is first released into the periarbuscular space (PAS), probably in the form of sucrose, then cleaved into hexoses and taken up by AM fungi through transport across the fungal plasma membrane. Within the fungal cytoplasm, hexoses are converted into glycogen granules and triacylglycerol (TAG) lipid droplets, which serve as suitable units for long-distance transport through the hyphal network. Nutrients that are acquired by the fungus from the soil and are delivered to the plant cell have to cross the fungal plasma membrane, be transported long distance to the intraradical hyphae (IRH), including the arbuscules, and subsequently reach the plant cytoplasm across the fungal plasma membrane and the plant periarbuscular membrane (PAM). Phosphate is imported by fungal phosphate transporters (cloned from *Glomus intraradices*[87] and *Glomus versiforme*[86]) that are present in extraradical hyphae (ERH). Phosphate is transported towards the root and IRH in the form of polyphosphate granules, which reside in membrane-enclosed vesicles. The negative charge of these granules makes them likely transport vehicles for metal ions and arginine. Phosphate is released from polyphosphate granules within IRH. Plant transporters that are involved in phosphate transport across the PAM have been cloned and characterized[80,101–103], whereas the fungal phosphate transporters that are responsible for the release of phosphate from IRH are still unknown. Nitrogen is taken up by ammonium[88], nitrate or amino-acid transporters in ERH. In AM fungal hyphae, nitrogen is mainly transported as arginine[106]. Within the IRH, nitrogen is released from arginine as urea and either transported to the plant directly or after cleavage to ammonium. Figure modified, with permission from REF. 8 (2003) American Society of Plant Biologists, REF. 90 (2005) Blackwell Publishing and *Nature* REF. 106 (2005) Macmillan Publishers Ltd. All rights reserved.

AM. This is still a developing area of research, but abscisic and jasmonic acid have emerged as potential regulators of AM[84,85].

AM Function

Nutrient uptake and transport in the extraradical mycelium. Fungal hyphae explore the soil substratum, but different AM fungi seem to use different strategies to do so (FIG. 3). The fungal hyphal network is ideally positioned to efficiently take up nutrients and water from the soil, but only a few fungal transporters that are involved in this process, including those that transport phosphate[86,87], ammonium[88] and zinc[89], have been cloned. Because diffusion is too slow, nutrients are moved in a packaged form between the extraradical and the intraradical mycelium (FIG. 7).

Carbon metabolism. Our understanding of the metabolic functions of the AM and AM fungi has been boosted by the development of axenic culture systems and the ability to restrict fungal and plant tissue to separate compartments, together with isotope labelling and *in situ* NMR (metabolism and transport in AM has been reviewed previously[90,91]). The plant can control the flux of sucrose directed to the root, including the fungus. Jasmonic acid has been proposed to be involved in the regulation of sink strength of AM roots[92]. Sucrose that is delivered to the AM root is cleaved either by symbiosis-induced sucrose synthases[93] or invertases[94]. *In vivo* NMR studies indicate that AM fungi obtain hexoses from the plant and convert them into lipids and glycogen for long-distance transport[8,95]. A member of the novel clade of hexose transporters that was identified from

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the symbiotic organ of the glomeromycotan *Geosiphon pyriformis* might also be expressed in the fungal interface membrane of the arbuscule[96]. In a typical AM, no carbon transport from the fungus to the plant was detected[97]. However, mycoheterotrophic plants that associate with AM fungi are likely to receive carbon from the fungus[26], and it is proposed that photosynthetic sporophytes of species of *Huperzia* deliver carbon to mycoheterotrophic gametophytes through shared fungal networks[98]. This would require carbon transporters that work in the efflux direction, which have not yet been reported in fungi. Alternatively the mycoheterotrophic plant must obtain carbon by efficiently digesting fungal hyphae, similar to the orchid symbiosis.

Phosphate. Improved phosphate uptake is the main benefit of the AM symbiosis[99,100]. The extensive hyphal network of AM fungi influences the physicochemical properties of the soil and directly or indirectly contributes to the release of phosphate from inorganic complexes of low solubility[6]. Fungal phosphate transporters that are expressed in the extraradical mycelium are probably involved in the uptake of phosphate from the substratum[86,87]. Polyphosphate granules are used as transport vehicles to move phosphate (and possibly arginine and trace elements) to the host root. Symbiosis-induced plant phosphate transporter genes have been identified in different plant species (reviewed in REF. 101), and accumulating evidence suggests a role for at least a subset of the corresponding proteins in symbiotic phosphate transport[80,102,103]. Fusions of potato *PT3* or *M. truncatula PT4* promoters to GUS targeted expression specifically to arbuscule-containing cells, which is consistent with results from laser-capture microdissection of tomato arbuscules, in which transcripts of five isoforms were detected in arbuscule-containing cells[104].

Nitrogen. AM fungi can accelerate decomposition and directly acquire nitrogen from organic material[105]. A fungal amino-acid transporter[46] and an ammonium transporter that might be involved in nitrogen uptake by extraradical hyphae have been cloned[88]. Long-distance transport to the plant probably proceeds mainly through arginine[106,107] (FIG. 7). Nitrogen is released in a carbon-free form (probably ammonium) to the plant[106], although the ammonium transporters in the symbiotic interface membranes have not yet been identified.

Evolution of plant root endosymbiosis

All members of the Glomeromycota phylum require a photosynthetic partner to complete their life cycle. Not a single member of this lineage has escaped from this dependency, which suggests that the ancestral fungus was already an obligate biotroph. This extreme specialization of an entire fungal clade is unique, as members of all phylogenetically comparable lineages — the ascomycetes, basidiomycetes and the non-monophyletic zygomycetes and chytridiomycetes (in the classical sense; for a more recent classification see REF. 4) — inhabit a wide range of ecological niches and include plant and animal pathogens and symbionts, as well as free-living saprophytes. AM is indeed an ancient symbiosis, and the excellent fossil record of early land plants from the Rhynie chert in Scotland provided 'rock-solid' evidence that typical AM fungal structures, such as arbuscules and spores, were already present 400 million years ago[108,109]. The high level of organization in these fossils and the wide distribution of AM in all branches of the phylogenetic tree of plants suggest that AM might have been present in a common ancestor and perhaps was instrumental in the initial colonization of land. Interestingly, the Rhynie chert fossils contain an impressive range of other fungal endophytes, including potential parasites[110], that provoked the formation of structural 'defences' by the plant, which reveals the ancient nature of symbiosis and defence programmes in land plants. Molecular-clock estimates of the age of members of the Glomeromycota differ by several hundred million years[111,112], but raise the possibility that they evolved before land plants. In this context, it is interesting that the glomeromycotan fungus *G. pyriformis* forms a symbiosis with photosynthetic (and nitrogen-fixing) species of *Nostoc* cyanobacteria and that similar fungus–bacteria interactions might have preceded the AM symbiosis[113]. Consistent with an ancient fungal-uptake mechanism for bacteria, most AM fungi harbour endosymbiotic bacteria, including Gram-negative *Burkholderia* species[114] and uncharacterized Gram-positive species[113], indicating multiple independent uptake events of symbiotic bacteria.

A conserved ancient genetic programme for AM

The presence of AM in the earliest land plants raises the possibility that the underlying genetic programme is conserved among extant AM-forming plants[115]. Indeed, phylogenetic and functional conservation of common *SYM* genes, at least in the angiosperm lineage, has recently been described[61,116]. Evidence from legumes indicates that the common symbiosis genes are required for the formation of the intracellular accommodation structure PPA[44]. This suggests that these genes are components of an ancient and conserved programme that evolved before the divergence of the angiosperms and was retained in most lineages because of the selective advantages conferred by AM.

AM is the ancestor of bacterial root endosymbioses. The discovery that some nodulation-defective legume mutants are also defective in AM development revealed a genetic link between bacterial and fungal symbiosis, which has led to the hypothesis that the root-nodule symbiosis evolved from AM functions. Now that common symbiosis genes have been cloned and functionally characterized from different nodulating and non-nodulating angiosperm species, we can draw a more detailed picture of the events that led to the evolution of the root-nodule symbiosis. The combined results suggest that the common symbiosis programme evolved in the context of AM and was recruited for the bacterial root-nodule symbiosis[115]. The identified genes are all required for induction of the intracellular accommodation programme, a common feature of both bacterial and fungal root endosymbiosis (FIG. 5). Most common *SYM* genes are conserved in overall domain composition between legumes and rice.

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A notable exception is SYMRK, which exhibits remarkable variation in its extracellular domain composition across angiosperms. Full-length SYMRK is consistently found in all tested members of the eurosid clade, whereas all other tested angiosperms contain shorter types that lack one of the leucine-rich repeats (LRRs), or one LRR and a long amino-terminal extension. A central role of SYMRK in the evolutionary events that led to nodulation was revealed by the observation that only full-length SYMRK can fully complement *Lotus symrk* mutants and restore nodulation, whereas shorter versions are sufficient only for AM[61]. This finding suggests that exon acquisition in an ancestor of the eurosids was associated with functional adaptation of SYMRK and provided the basis for bacterial triggering of the common symbiosis programme by bacteria[61]. In a hypothetical scenario, SYMRK evolution was a prerequisite for an intracellular symbiosis with nitrogen-fixing bacteria that was manifested by the formation of intracellular infection threads. Such an early bacterial symbiosis might not have been associated with nodule organogenesis[115]. A situation that mimics such early bacterial endosymbiosis is found in the *hit1/har1* double mutant of *L. japonicus*, which develops abundant infection threads in the absence of nodule formation[117].

Phylogenetic and functional analysis of the symbiosis receptor kinase gene *SYMRK* has revealed functional and structural polymorphism across angiosperms, which suggests that this gene had a key role in the evolution of the nitrogen-fixing root-nodule symbiosis on the basis of a pre-existing AM genetic programme.

Because many of the important plant pathogenic fungi share an intracellular biotrophic lifestyle with AM fungi, it has been suggested that both parasitic and symbiotic fungi rely on partially overlapping intracellular accommodation programmes of the plant[118]. However, *L. japonicus* mutants that are defective in common symbiosis genes support completion of the life cycle of a leaf rust fungus, including the formation of intracellular haustoria[119]. So far, plant genes that support biotrophic fungal pathogens are largely unknown, and therefore the molecular components of such a shared programme have been elusive.

Conclusions and outlook

Over the past few years, a novel and unexpected developmental capacity of plant cells has been discovered that is essential for the intracellular uptake of AM fungi. Plant genetics will continue to be a major tool in the identification of genes that are required for AM development and function. It is expected that in the near future, the chemical structure of the fungal Myc factor that triggers the symbiotic responses of the root will be published, which will help us to identify the cognate plant receptors. To unlock the potential of AM for sustainable agriculture, we must identify the key molecular players. Equally importantly, we must investigate the natural variation for AM function and responsiveness within biodiversity collections of important crop plants[120] and between different fungal lineages. The long-term aim is to identify or design crop–fungus combinations with optimized AM performance, which would be instrumental in reducing the application of fertilizer and energy input, a goal that is mandatory in a world of depleting non-renewable resources[121].

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Acknowledgements
I thank A. Schüßler for helpful discussions and for adapting FIG. 1, and K. Haage for compiling TABLE 1. I apologize to all those colleagues whose work was not cited owing to space restrictions.

DATABASES

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Lotus japonicus | *Medicago truncatula* | *Mesorhizobium loti* | *Sinorhizobium meliloti*

FURTHER INFORMATION

Martin Parniske's homepage:
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