The arbuscular mycorrhizal (AM) symbiosis is one of the most widespread symbiotic associations found in plants, yet our understanding of events underlying its development are limited. The recent integration of biochemical, molecular and genetic approaches into analyses of the symbiosis is providing new insights into various aspects of its development. In the past year there have been advances in our understanding of the signals required for the formation of appressoria, the molecular changes in the root in response to colonisation, and components of the signal transduction pathways common to both the AM and Rhizobium symbioses.

Introduction

Over 80% of vascular flowering plants are capable of entering into symbiotic associations with arbuscular mycorrhizal (AM) fungi, and in natural environments the roots of many plants are really symbiotic organs termed mycorrhizae. The fungi that form these associations are members of the zygomycetes and the current classification places them all into one Order, the Glomales [1]. The AM association is a relatively non-specific, highly compatible, long lasting mutualism from which both partners derive benefit. The plant supplies the fungus with carbon, on which it is entirely dependent. The fungal contribution is more complex—it is clear that the fungus assists the plant with the acquisition of phosphate and other mineral nutrients from the soil, and it is also apparent that it may influence the plant's resistance to invading pathogens [2]. In addition to its ecological significance, the association may also have applications in agriculture, particularly in soil conservation, may be fully exploited.

Development of the symbiosis is associated with significant alterations in the interior morphology of the root cortex and in the physiology of the plant. These aspects have been reviewed elsewhere in detail [4-7]. In brief, the interaction begins when fungal hyphae, arising from spores or from adjacent colonised roots, contact the root surface. Here they differentiate to form appressoria via which they penetrate the root (Figure 1). Once inside the root, the fungus may grow both inter- and intra-cellularly throughout the cortex but does not invade the vasculature or the meristematic regions. The types of internal structures that develop depend on the plant/fungal combination and may include intracellular, differentiated hyphae called arbuscules and/or intracellular coils [8]. Although the fungal hypha penetrates the cortical cell wall to form the arbuscule within the cell, it does not penetrate the plant plasma membrane and this extends to surround the arbuscule (Figure 1). In addition to internal growth within the root, the fungus also maintains external mycelia which ramify out into the soil. These external hyphae access phosphate which is then transported to the internal structures and eventually released to the root. The interface between the fungal arbuscule and the cortical cell is probably important for nutrient transfer between the symbionts, but this has not yet been demonstrated directly [9]. In comparison to other plant/microbe interactions, we still know relatively little about the molecular events underlying development of the AM symbiosis. The obligate, biotrophic nature of the fungi has contributed to the difficulties with molecular and genetic analyses of these associations and it is only recently that such approaches have been applied. This review surveys the most recent molecular genetic and biochemical studies of the association and their contributions to basic knowledge of the AM symbiosis.

Signals for the development of the AM symbiosis

The formation of the symbiosis requires the co-ordinate development of both the fungus and the plant and is assumed to require an interchange of signals. As AM fungal spores are capable only of germination and limited hyphal growth in the absence of the plant, it seems likely that plant signals are essential for the initial stages of the symbiosis. Root exudates have been shown to stimulate growth and branching of the AM fungal hyphae and low concentrations of certain flavonoid/isoflavonoid compounds, which are major components of some plant root exudates, are also capable of promoting hyphal growth [10,11]. Recent data suggest that AM fungi have a receptor for the flavonoid/isoflavonoid compounds. Flavonoids, particularly isoflavonoids, are known to have estrogenic activity and the estrogen 17β-estradiol was shown to stimulate AM fungal hyphal growth in a similar manner to that of the isoflavonoid biochanin A, while antiestrogens competitively inhibited the biochanin A-mediated growth stimulation. The data are consistent with the presence of a fungal receptor in which the A and C rings of the flavonoid and the hydroxyl group at position A-7...
are likely to be important features for recognition of the molecule ([12]; Figure 2). Despite the evidence that flavonoid/isoflavonoids promote growth of these fungi it seems unlikely that they are essential for development of the association, as maize mutants lacking flavonoids are still able to form the symbiosis [13]. In addition, isoflavonoids are unlikely to be a universal signal, as they are found only in a subset of the plants capable of forming the symbiosis. Instead, such compounds probably signal the presence of a root and the fungus can respond by increasing hyphal growth and branching to enhance the possibility of contacting the root.

On contact with the root, the fungus differentiates to form appressoria on the surface of the epidermal cells. These structures have only been observed on plant roots and they do not form on synthetic surfaces even in the presence of growth-stimulating exudates [10,14]. Recently it was demonstrated that the mycorrhizal fungus, *Gigaspora margarita* was able to form appressoria on purified carrot epidermal cell wall fragments [15*]. These elegant experiments indicate that the cell wall alone is sufficient to stimulate formation of appressoria and signals secreted from the roots are not necessary. In addition, the cell walls of the non-host, sugar beet, do not permit appressoria development and, therefore, topology of the epidermis is not sufficient to induce appressoria and endogenous signals must be present in host wall. This is supported further by observations that appressoria form only on epidermal cell wall fragments and not on cell walls from other parts of the root such as the cortex or vasculature. The appressoria that form on the host cell wall fragments do not develop complete penetration hyphae which suggests that the next stage of development requires an intact cell [15*]. Thus the epidermal cell wall signals appressoria formation; the next task will be to elucidate the specific cell wall component(s) that trigger the response.
Following the formation of appressoria, the fungus penetrates the root and proceeds to colonise the cortex and differentiate to form arbuscules. Plant mutants on which the fungus is able to form appressoria but unable to penetrate the root have been identified in a number of legumes [16–19]. Some of these mutants may be lacking a signal for entry and the cloning of the mutated genes has the potential to provide insight into signalling at this stage of the symbiosis. The nature of the signals involved in the later stages of the association is entirely unknown; however, it is conceivable that the more common secondary metabolites are involved. Flavonoids increase in mycorrhizal legume roots and sesquiterpenoid cyclohexenone derivatives were shown recently to accumulate during mycorrhizal development in many members of the Poaceae [20,21,22]. Other possible candidates for later signals include hormones. Induction of cytokinins in mycorrhizae has been reported previously and this was confirmed recently in alfalfa, where significant induction of ZR (trans-zeatin riboside) type cytokinins was observed in mycorrhizal roots [23,24]. At the moment, signals derived from the fungus are entirely unknown.

**Molecular events associated with the development of the mycorrhiza**

The identification of plant mutants unable to form a complete symbiosis indicates that development of the association is controlled, at least in part, by the plant. The nature of this control, however, is currently unknown [16]. There has been recent progress in the identification of genes showing differential expression in mycorrhizal roots and although it will require extensive analyses to determine whether these genes have a significant role in the symbiosis, they currently provide information on the molecular events occurring during formation of the association. In addition they may serve as molecular markers for different developmental stages in the symbiosis. A mycorrhizal inducible β-tubulin gene was identified in maize and expression of promoter–reporter gene fusions in tobacco indicated that this gene is induced in the cells in which arbuscules are developing [25]. This is consistent with alterations in the cytoskeleton which occur as the internal structure of the cell is reorganising to accommodate the arbuscule and to develop the peri-arbuscular membrane (Figure 1). The cell wall of the arbuscule and the peri-arbuscular membrane that surrounds it are separated by a narrow interface compartment that is continuous with the plant cell wall although it differs in structure ([26]; Figure 1). Antibody probes and stains have revealed that the compartment is a complex mixture of molecules including xyloglucans, arabinogalactans, β-D-1→4 glucans and hydroxy rich glycoproteins [27–28]. The significance of this molecular composition is currently unknown but it has been speculated that some of the components may act as signals [5]. Potential candidates might include small oligosaccharides or arabinogalactan proteins, both of which are known to act as signaling molecules in other systems. The importance of cell wall signals in the early stages of the AM symbiosis [15•] indicates that these types of signals should not be overlooked.

One of the major alterations in the cortical cells in which the arbuscules form is the massive extension of the plasma membrane to form the peri-arbuscular membrane ([29]; Figure 1). Given that the exchange of nutrients is assumed to occur at this interface, it might be expected that membrane transport mechanisms will be induced. Consistent with this suggestion, a cDNA representing a mycorrhizal inducible plasma membrane ATPase was identified recently in barley [30] and a novel gene, predicted to encode a membrane anchored protein with structural similarities to phospholamban, a vertebrate protein that regulates the activity of a Ca2+-ATPase, is induced in mycorrhizal pea [31]. The induction of an ATPase gene is consistent with biochemical data indicating increases in plasma membrane ATPase activity in mycorrhizal roots of some species [32] and with earlier studies that demonstrated high levels of ATPase activity on the peri-arbuscular membrane [33]. The putative function of ATPases on this membrane is to provide energy for nutrient transfer processes; however, transport proteins involved in nutrient movement at the arbuscular interface are currently unknown. A mycorrhizal inducible sugar transporter has been identified in Medicago truncatula [34] and a gene encoding a member of the membrane intrinsic protein gene family is induced in parsley mycorrhizae. Members of this family have been shown to facilitate the movement of water and other small molecules [35].

Phosphate regulates the symbiosis between AM fungi and plants and the extent of colonisation in the roots is inversely correlated with the phosphate status of the plant [36,37]. Since the symbiosis usually results in increased levels of phosphate in the plant some of the plant genes showing differential expression in mycorrhizal roots may be regulated in response to phosphate, rather than signals from the fungus. There is, however, evidence for independent regulation of expression of one gene, Mt4, via both of these stimuli. Mt4 is a novel cDNA representing a root specific gene from M. truncatula [38•]. Mt4 transcripts are induced in response to phosphate
starvation and down-regulated by phosphate or in response to colonisation by a mycorrhizal fungus. It was initially assumed that down regulation in the symbiosis was a consequence of increased phosphate; however, Mr4 expression is also down regulated in a symbiotic plant mutant in which colonisation is limited to growth on the surface of the root and in which phosphate transfer does not occur. This suggests that the Mr4 gene is regulated independently by two signals, phosphate and an unknown component(s) of the mycorrhizal fungus. Two phosphate transporters and an acid phosphatase show similar patterns of expression in response to phosphate starvation and colonisation by mycorrhizal fungi, but it is unknown whether they also show independent regulation in response to the two signals [39]. Together, these data indicate that, in general, phosphate starvation inducible genes are down regulated in the early stages of the symbiosis, ahead of significant levels of phosphate entering the root. Has the symbiosis co-evolved to the extent that the plant ‘anticipates’ an increased supply of phosphate before it is delivered? Certainly the symbionts have co-existed for the extensive periods of time that might be necessary for this to occur. Fossil evidence indicates that arbuscular mycorrhizae were present in Devonian land plants and thus plants and AM fungi have been associated for over 400 million years [40].

In comparison with the plant, even less is known about the molecular changes in the fungal symbiont as the association develops. The recent identification of genes expressed in the spores [41] coupled with the continuing identification of fungal genes expressed during the symbiotic phase of the life cycle [42,43] will be essential in unravelling aspects of AM fungal development.

Conserved signalling pathways in the Rhizobium-legume and mycorrhizal symbioses

It has been speculated that the Rhizobium–legume (bacterial–plant) symbiosis evolved from the much older mycorrhizal (fungal–plant) symbiosis and emerging similarities in the molecular events occurring during their development lend support to this idea [44]. The observation that a number of nodulation mutants are also mycorrhizal mutants indicates the presence of genes essential for both symbioses; however, the identity of the genes responsible is unknown [16–19]. Recent studies indicate that a number of the nodulin genes induced during nodule development are also induced in the mycorrhizal symbiosis, although their function in the latter is unclear. In Vicia faba, one of the four leghaemoglobin genes induced in nodules is also induced in mycorrhizal roots [45] and three other nodulin genes, PsENOD12, PsENOD2 and MsENOD40 are induced in mycorrhizal pea and alfalfa roots, respectively [5,24*]. In mycorrhizal alfalfa roots, MsENOD40 transcripts are localised to the pericycle, epidermis and cortex as seen in roots inoculated with Rhizobia, and co-localise with cells containing immature arbuscules. The MsENOD2 transcripts are present in cells containing mature arbuscules, whereas in nodules these transcripts are localised in the nodule parenchyma. Induction of MsENOD2 and 40 occurs in response to cytokinin, which is elevated in roots during nodulation and has been shown recently to increase in mycorrhizal alfalfa roots [24*]. On the basis of these data the authors suggest that the downstream signalling events are conserved between the two symbioses; however, it is equally possible that the similarity lies only in the increase in cytokinin and the pathways prior to cytokinin induction are distinct.

Further support for conserved signalling pathways is provided by studies of PsENOD5 and 12A expression in mycorrhizal and nodulated pea and pea mutants [46*]. As in nodule development, PsENOD5 and PsENOD12A are induced in the early stages of the pea mycorrhizal symbiosis. Further experiments using the pea sym8 mutant, which is blocked in early stages of both nodulation and mycorrhiza formation, demonstrated that a functional Sym8 was necessary for expression of the downstream PsENOD5 and PsENOD12A genes in both symbioses. Sym 8, therefore, is a common step in the signal transduction pathways for both of these symbioses. It seems unlikely that the initial signals for the two symbioses will be the same; however, at some point—probably shortly after the initial signal—the signal pathways converge and a number of downstream events are conserved.

Conclusions

As the application of molecular approaches to analyses of this symbiosis is relatively recent, it can be anticipated that the first few years will include groundwork to support future advances. In the past year molecular approaches have contributed to our understanding of some aspects of the symbiosis and generated molecular tools essential for future analyses. Thus, the end of the lag phase is approaching and the next few years should see a rapid increase in our understanding of the events underlying the AM symbiosis. The identification of mycorrhizal mutants is accelerating and the continued cloning of genes induced during the symbiosis will contribute to a molecular picture of the events accompanying development of the association. These genes will also serve as tools; both for the analysis of altered developmental processes in the mutants, and as a starting point for the analysis of signal transduction pathways operating in the symbiosis. The symbiosis is a complex system and it will be important to use integrated approaches to obtain a complete understanding of the events underlying its development and functioning.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
• of outstanding interest


8. Smith FA, Smith SE: Structural diversity in (vesicular)-

A detailed and informative review of the diversity of structures present in arbuscular mycorrhizal symbiosis with discussion of their significance with respect to plant/fungal interfaces involved in nutrient transport. This is particularly important; the presence of different types of structures tends to be overlooked because the majority of experimental work has involved plants forming the Arum-type mycorrhiza.


15. Nagahashi G, Douds DD Jr: Appressorium formation by AM fungi on isolated cell walls of carrot roots. New Phytol 1997, 136:299-304. Elegant experiments demonstrating that the plant signal that induces appressorium formation by AM fungi resides in the epidermal cell wall of host plants. Isolated epidermal cell walls were a suitable substrate for appressorium formation, however, penetration hyphae did not develop, suggesting that subsequent stages require the presence of an intact cell.


25. Elevated levels of cytokinin and the induction of MsENOD2 and MsENOD40 were observed in alfalfa roots following colonisation by mycorrhizal fungi. The data are consistent with the conservation of signal transduction pathways in the nodulation and mycorrhizal symbioses.


Development of the arbuscular mycorrhizal symbiosis


46. Albrecht C, Geurts R, Lapeyrie F, Baseling T: *Endomycorrhizae* and rhizobial nod factors activate signal transduction pathways inducing *PsENOD5* and *PsENOD12* expression in which Sym8 is a common step. *Plant J* 1998, in press.

This paper provides direct evidence for signal transduction pathway components shared in the Rhizobium and mycorrhizal symbioses. In pea, *PsENOD 5* and *PsENOD 12A* are induced in the early stages of both symbioses. Studies with symbiotic mutants indicate that these genes are not induced in the *sym8* mutant. Thus a functional Sym8 gene product is required for the induction of *PsENOD 5* and 12A expression in both symbioses and represents a common component of the signalling pathways.