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Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas

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Abstract

Bidirectional nutrient transfer between the plant and the fungus is a key feature of arbuscular mycorrhizal symbiosis. The major nutrients exchanged between the symbiotic partners are reduced carbon, assimilated through the plant photosynthesis and phosphate, taken up by the fungal hyphae exploring soil microhabitats. This nutrient exchange takes place across the symbiotic interfaces which are bordered by the plant and fungal plasma membranes. This review provides an overview of the current knowledge of the mechanisms underlying nutrient transport processes in the symbiosis, with special emphasis on recent developments in the molecular biology of the plant and fungal primary (H^+-ATPases) and secondary transporters.

Introduction

The mutualistic nature of arbuscular mycorrhizal (AM) symbioses relies on the ability of the fungal mycelium to take up mineral nutrients from the soil solution and to transfer them to the symbiotic roots in exchange for carbohydrates. Plant benefits from AM symbiosis are traditionally recognized as improved access to limiting soil resources, mainly immobile nutrients such as P, Cu, Zn and ammonium. On the other hand, the carbon supplied from the host plant to the fungus is essential to the formation and functioning of arbuscular mycorrhizas and for the completion of the fungal life cycle. Although the mutualism of the AM association is based on this bidirectional nutrient exchange, this does not necessarily mean that nutrient transfer from the fungus to the plant is directly linked to C transfer to the fungus. In fact, different AM fungi in symbiosis with the same host plant, can considerably differ in their C–P exchange ratio and, consequently, in their degree of functional compatibility with the plant (Pearson and Jakobsen, 1993). Additionally, several studies have shown that C transfer can be decoupled from P transfer under certain environmental conditions, as for instance, under conditions of high soil P availability (Graham et al., 1997). In any case, an indirect link between C and P transfer is evident as C supplied to the fungus will support mycelia growth and, indirectly, P uptake from the soil.

This bidirectional transfer of nutrients between the plant and the fungus is believed to take place across the symbiotic interfaces which are bordered by the plant and fungal plasma membranes (Smith and Smith, 1990). Although current knowledge of the molecular mechanisms underlying transport nutrient in arbuscular mycorrhizas is still in its infancy, it is generally accepted that such bidirectional transfer of nutrients across the symbiotic interfaces involves passive efflux of solutes from the donor organism into the interfacial apoplast, followed by active uptake by the receiver organism (Smith and Read, 1997; Woolhouse et al., 1975). A major obstacle to the study of these mechanisms is the difficulty of culturing the fungal partner in the absence of the host plant and of isolating the intact arbuscules that are developed within the cortical root cells. The recent application of molecular tools to the study of the AM symbiosis is enabling the identification of important transport systems, and the results obtained so far support the existence of active mechanisms involved in the transfer of carbon and phosphate, as hypothesized by Woolhouse (1975). This review

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focuses on current knowledge about the molecular characterization and regulation of transport proteins located in the plant and fungal plasma membranes. The data are discussed in relation to the mechanisms underlying nutrient transport processes in arbuscular mycorrhizas.

Structure and composition of AM symbiotic interfaces

It is widely accepted that in AM symbiosis, as in other biotrophic interactions, the exchange of nutrients between the plant and the fungus takes place across the symbiotic interfaces that are developed during the colonization process of the host plant root (Smith and Smith, 1990). Due to the considerable importance of the symbiotic interfaces for nutrient transport in AM symbiosis, a brief revision of the current knowledge about the structure and composition of the symbiotic interfaces is presented below.

Two different types of symbiotic interfaces can be found, depending on whether the fungus grows inter- or intracellularly in the root system. Intercellular interfaces are created when the fungal hyphae grows within the intercellular spaces of the root cortex, whereas intracellular interfaces are developed when the fungal hyphae penetrates the wall of the root cells. Intracellular structures include coils of fungal hyphae, often formed during initial colonization of the epidermis and (if present) hypodermis in many AM roots, and especially in cortical cells of *Paris*-type mycorrhizas, and arbuscules which are the main intracellular structures in *Arum*-type mycorrhizas (Smith and Smith, 1997). Since very little work has been done on the physiology and molecular biology of *Paris*-types, this chapter focuses on *Arum*-types.

Both inter- and intracellular interfaces have the same basic structure, they are always composed of the membranes of both symbionts separated by a narrow interfacial space. However, there are significant differences among them, concerning mainly to the composition and structure of both plant and fungal walls and to the presence of other materials found in the interfacial apoplast (for extensive revision, see Bonfante and Perotto, 1995; Bonfante, 2001).

When root cells are colonized by AM fungi to form arbuscules the host plasma membrane invaginates and proliferates around the developing fungus. Alexander et al. (1989) have reported a 3.7-fold increase of host plasmalemma in arbuscule-containing cells. The host plasma membrane surrounding the arbuscules is termed periarbuscular membrane and its formation must involve massive synthesis of lipids and proteins, some of the latter being unique to this membrane. The difficulty of isolating the periarbuscular membrane has hampered its biochemical characterization; however, Benabdellah et al. (2000) have shown by 2D-PAGE analysis of plasma membrane proteins isolated from mycorrhizal and non-mycorrhizal roots that development of the symbiosis induces the synthesis of at least 10 new plasma membrane polypeptides. In addition, the amount of phospholipids is higher in membranes isolated from mycorrhizal roots (Benabdellah, 1999). The application of efficient cytological and molecular tools has circumvented the difficulties of biochemical approaches and is enabling the characterization of the periarbuscular membrane. Cytochemical and immunocytochemical analysis have shown that the periarbuscular membrane displays ATPase activity, which is usually absent in the peripheral membrane of the colonized cortical root cell, suggesting that this membrane acquire transport functions that are usually absent in the cortical root cells (Gianinazzi-Pearson et al., 1991, 2000). The nature of other proteins in this membrane remains unknown, although immunocytological studies have shown that the periarbuscular membrane shares components with the peribacteroid membrane in the *Rhizobium*–legume symbiosis (Perotto et al., 1994).

In situ use of affinity probes, such as enzymes, lectins or antibodies has shown that the interfacial apoplast between the periarbuscular membrane and the fungal surface presents many molecules typical of the primary cell wall, such as cellulose, β-1,4-glucans, polygalacturans and hemicelluloses (Bonfante and Perotto, 1995; Gollotte et al., 1997). However, morphological analysis of the interface material indicates that these components are not assembled into a fully structured cell wall (Bonfante and Perotto, 1995). Recent *in situ* hybridisation experiments have shown that activation of some genes in arbuscule-containing cells such as those encoding proteins rich in hydroxyproline (Balestrini et al., 1997), arabinogalactan proteins (van Buuren et al., 1999) and xyloglucan endotransglycolases (Maldonado-Mendoza and Harrison, 1998) might account for the composition and structure of the interfacial matrix.

In relation to the fungal cell wall at the arbuscular level, ultrastructural studies have shown that it is very thin around arbuscule branches and that the chitin, the main component of AM fungal cell wall, has at this

level an amorphous nature, in contrast with the rigid and fibrillar structure that presents in the intercellular hyphae (Bonfante-Fasolo et al., 1990). Moreover, these studies have shown that the plant cell wall at the intercellular interfaces suffers little modifications. The observations derived from all these studies, and the fact that arbuscules increase the contact area between symbionts, support the general assumption that the transfer of nutrients takes place preferentially across the arbuscular interfaces (Smith and Smith, 1990). However, some authors have proposed that the presence of other AM interfaces might offer the possibility that different compounds are transferred across the various interfaces (Smith and Read, 1997). Although hyphae and hyphal coils have been usually ignored as potential transfer sites, there is no reason to assume that they do not have transfer functions. The recent observation by confocal microscopy that the intracellular coils in *Paris*-type AM present as great surface area per cortical cell as an arbuscule in *Arum*-types opens the possibility that intracellular coils might have some relevance in nutrient transfer (Dickson and Kolesik, 1999). The possible role of intercellular hyphae in carbohydrate uptake will be discussed in the next section.

It is not clear how far the wall and wall-like materials present in the interfacial apoplast are involved in, or influence, transfer of nutrients across the interface. However, it is likely that nutrient movement between partners is mainly controlled by the bordering plant and fungal plasma membranes, and more specifically by the plasma membrane proteins that mediate controlled exchange of solutes into and out of the adjacent cells. Therefore, elucidation of the mechanisms underlying the controlled transfer of nutrients between the mycorrhizal symbionts requires identification of the specific transport proteins involved in efflux and uptake of solutes by both partners. These membrane proteins have not so far been identified; however, the application of molecular biology techniques to the study of arbuscular mycorrhizas is providing some insights about the transport processes that occur in the symbiotic interfaces. As in other organisms, major advances in the understanding of these processes across the partner membranes have emerged from recent studies of the dominant H^+-pumping ATPase and from the identification of a small range of secondary transporters.

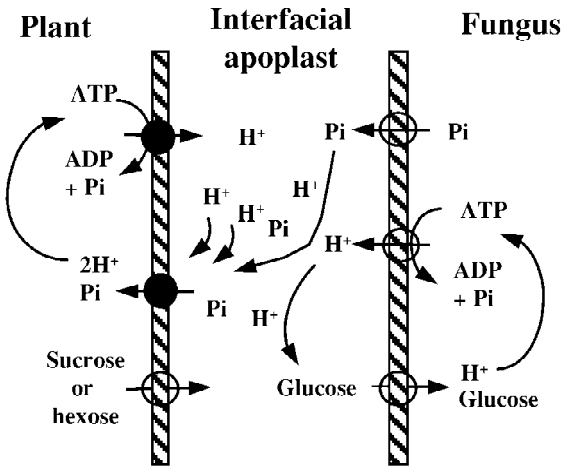

Figure 1. Schematic illustration of the proposed model for the transfer of phosphate and carbon compounds across the arbuscular interface. Plasma membrane H^+-ATPases and secondary transporters that have been experimentally localized in the membranes of the arbuscular interface are indicated by filled circles.

Carbon supply to the fungus

AM fungi, as obligate symbionts, depend for their growth and activity on the supply of carbon compounds by the photosynthetic partner (Jennings, 1995). Consequently, AM symbiosis can cause an important carbohydrate drain: up to 20% of total photoassimilate production can be transferred to the fungal partner (Douds et al., 2000; Graham et al., 2000). There is enough evidence that, as a consequence of mycorrhizal establishment, assimilates are directed towards the root and the rate of net photosynthesis of the host increases (Douds et al., 1988; Kucey and Paul, 1982; Nemec and Vu, 1990; Wright et al., 1998). The fact that cells containing arbuscules are acting as a sink for sucrose is supported by *in situ* hybridisation studies of the expression of genes involved in sucrose hydrolysis such as sucrose synthase and soluble acid invertase (Blee and Anderson, 1998).

Studies using isotopic labelling with nuclear magnetic resonance spectrometry in AM roots (Shachar-Hill et al., 1995) and radiorespirometry measurements on isolated intraradical hyphae (Solaiman and Saito, 1997) have shown that the internal phase of the fungal symbiont can take up and use hexoses, mainly glucose. However, it is still a matter of debate whether the transfer occurs across the intercellular or the intracellular interfaces and how the transfer occurs. Although it has not been experimentally proved, it is generally assumed that the transfer of carbon compounds from

the plant to the fungus involves passive efflux across the plant plasma membrane followed by active uptake by the fungal plasma membrane from the interfacial apoplast (Figure 1).

The nature of the membrane transport proteins that mediate these processes remains still unknown. A cDNA clone encoding a hexose transporter whose expression increases in cells containing arbuscules, as well as in adjacent cells, has been cloned in *Medicago truncatula*. Functional characterization of the protein encoded by this gene demonstrated its ability to take up glucose by a H^+ symport process, suggesting that this protein is not involved in the efflux of glucose across the plant membrane at the symbiotic interface (Harrison, 1996). Additionally, a cDNA encoding a novel sugar transporter has been recently cloned in tomato roots following a RT-PCR approach (Ferrol, unpublished results). Preliminary gene expression analysis revealed that this gene was constitutively expressed in tomato roots and that the sugar transporter transcripts increase in leaves of wild-type plants but not in those of mycorrhiza-defective tomato mutants after inoculation with *Glomus mosseae*. Since, as stated before, carbon partitioning increases in mycorrhizal plants, these observations suggest that this transporter could play a role in the symbiosis by increasing the loading into the phloem of the reduced carbon compounds demanded by the microsymbiont for its growth and activity and/or by the increased metabolic activity of arbuscule-containing cells. Ferrol et al. (2002) have also found that development of a successful mycorrhizal interaction increases leaf H^+-ATPase gene expression. This further supports the possibility that phloem loading increases in mycorrhizal plants, since active loading of carbon compounds into the phloem requires the action of a plasma membrane H^+-ATPase (DeWitt et al., 1991; Villalba et al., 1991). Summarising, although sugar transporters that are regulated by the symbiosis have been identified, the plant transporter involved in carbon efflux across the host plasma membrane remains unidentified.

With regard to the mechanisms by which AM fungi take up carbon compounds from the apoplastic interface, it is unclear whether uptake requires an active transport system or whether concentrations of carbon at the interfaces are sufficient to direct uptake by facilitated diffusion. In the ectomycorrhizal fungus *Amanita muscaria*, a hexose transporter that operates by an active hexose-H^+ symport process seems to be responsible for the sugar uptake by the fungus (Nehls et al., 1998; Wiese et al., 2000). Since membranes involved in active solute uptake by H^+ co-transport must be energized by a H^+-ATPase (Palmgren and Harper, 1999; Serrano, 1989), the presence of ATPases in fungal membranes could provide clues about the mechanisms of transport and the sites of glucose transfer. Based on the cytochemical observation that the membrane of the arbuscular branches frequently lacks ATPase activity while the membranes of the intercellular hyphae show high levels of ATPase staining, it was suggested that carbon might be transferred across the intercellular interfaces rather than across the arbuscular interfaces (Gianinazzi-Pearson et al., 1991). However, as pointed out by these authors, this hypothesis must remain tentative until more clear evidence on fungal H^+-ATPase distribution is obtained by other methods. Five PCR fragments encoding plasma membrane H^+-ATPases have been identified in *Glomus mosseae* (Ferrol et al., 2000) and two in *Glomus intraradices* (Ferrol et al., unpublished data). RT-PCR expression analysis revealed that one of the *G. mosseae* genes was expressed in the intraradical structures of the fungus, indicating that active transport processes occur in these structures; however, the specific location of these transcripts remains to be determined (Ferrol et al., unpublished data). Although these studies on H^+-ATPases are providing some insights into the transport mechanisms operating at the fungal membranes, knowledge of the particular mechanism by which AM fungi take up carbon compounds from the apoplastic interface and about the interfaces involved requires the identification and location of the membrane proteins involved.

Transfer of phosphate from the fungus to the plant

Root colonization by the AM fungus is accompanied by the development of a network of fine extraradical hyphae, which increases the absorption rate of slow-diffusing nutrients, mainly phosphate (P), from the soil to the plant (Jackobsen, 1999). Movement of phosphate in the symbiosis begins with its uptake from the soil solution by the phosphate transporters located in the extraradical hyphae, followed by its translocation to the intraradical fungal structures and subsequent transfer to the colonized root cell across the arbuscular interfaces.

Molecular cloning of a phosphate transporter from *Glomus versiforme* has provided evidence about the mechanisms of phosphate uptake by the external hyphae (Harrison and van Buuren, 1995). Functional

characterization of this gene in yeast cells indicated that it encodes a high-affinity transporter that operates by an active H^+ symport process. The finding that the transporter transcripts were present in the external mycelium, but not in the intraradical fungal structures, led Harrison and van Buuren (1995) to propose that this transporter may be responsible for the initial uptake of phosphate into the mycorrhiza. Functioning of active secondary transporters requires the action of the plasma membrane H^+-ATPase (Serrano, 1989), therefore, this protein must be present on the plasma membrane of the extraradical hyphae. In this context, Ferrol et al. (unpublished data) have found that H^+-ATPase genes are expressed in the extraradical hyphae of *G. mosseae* and *G. intraradices*. These observations indicate that uptake of P by the extraradical hyphae is mediated by a high-affinity transporter whose ability to function depends on the electrochemical gradient of H^+ generated by the plasma membrane H^+-ATPase.

Uptake of P by extraradical hyphae is followed by the synthesis of large amounts of polyphosphate, part of which is stored in the fungal vacuoles (Cox et al., 1980; Dexheimer et al., 1996). A recent study using *in vivo* ^{31}P nuclear magnetic resonance has shown that the phosphate ions taken up by the fungus are transformed into polyphosphate with a short chain length (Rasmussen et al., 2000). Based on these observations the authors suggest that the process of phosphate translocation along the hyphae of AM fungi may be similar to that of ectomycorrhizal fungi, in which the phosphate appears to be transported mainly as polyphosphate in a motile vacuole system. This is assumed to be followed by hydrolysis of polyphosphate by the action of the phosphatases that have been localized in the vacuoles of the intraradical hyphae (Ezawa et al., 1995; Jabari-Hare et al., 1990; Tisserant et al., 1992) and exopolyphosphatases (Ezawa et al., 2001).

The P transfer across the symbiotic interface begins with efflux from the fungus into the interfacial apoplast, an unusual step, since membrane transport processes for P transport favour absorption over loss (Clarkson, 1985). Several hypothesis have been proposed to explain the high P loss from the arbuscule (Harley and Smith, 1983, Smith and Read, 1997). As individual arbuscules have a lifespan of about 7–11 days, it was accepted in the past that its senescence and partial breakdown would release P to be taken up by the host plant. Nevertheless, measurements of P flux rates across the interface showed that the release of P from senescing arbuscules would be far too slow to account for P transfer to the host (Cox and Tinker, 1976). Nor it is likely that increased leakiness of P by simple diffusion as a result of an increased membrane permeability would support the massive P transfer in AM symbiosis. Consequently, it is believed that transport of phosphate across the arbuscular membrane probably involves specific efflux channels or transporters.

Recent molecular studies of host plasma membrane H^+-ATPases and phosphate transporters have provided evidence that the subsequent uptake of phosphate by the plant may be an active transport process. Up-regulation of host plasma membrane ATPase genes has been demonstrated in barley, tobacco and tomato plants (Ferrol et al., 2002; Gianinazzi-Pearson et al., 2000; Murphy et al., 1997). In tobacco roots, two H^+-ATPase genes were found to be induced in arbuscule-containing cells, by using promoter β-glucuronidase (GUS) fusions (Gianinazzi-Pearson et al., 2000). In addition, immunocytochemical analysis of these roots detected the H^+-ATPase protein in the periarbuscular membrane. Recently, Rosewarne et al. (1999) have identified a tomato phosphate transporter that may be involved in the uptake of the phosphate that is effluxed across the arbuscular membrane, as indicated by the high transcript levels detected by *in situ* hybridisation in arbuscule-containing cells. Based on these observations, it can be concluded that the periarbuscular membrane plays an important role in the absorption by the host plant of the phosphate ions that have been transported by the fungus and released into the symbiotic interface through a H^+-phosphate symporter activated by the plasma membrane H^+-ATPase (Figure 1).

Conclusions

Transport processes are crucial for the functioning of arbuscular mycorrhizal symbioses because they are nutritionally based. The picture of how carbon compounds and mineral nutrients are transferred between the symbionts will be clarified once the major transport proteins had been cloned and located at the interfacial membranes. This will also contribute to a better understanding of the mechanisms regulating AM functioning. During the last years, the application of techniques of molecular biology to mycorrhizal research has brought some information about the molecular mechanisms underlying phosphate uptake by the external hyphae and the periarbuscular membrane. Until now, all the transporters that have been shown

or postulated to be involved in the functioning of the symbiosis are proton symporters, which emphasizes the importance of H^+-ATPases for nutrient uptake. From this review it should be evident that our knowledge about transport mechanisms and the molecular structure of the plant and fungal transporters is, at best, scarce. There is in consequence a clear need for further work on the isolation and molecular characterization of these membrane proteins in order to understand the mechanisms underlying nutrient transport processes in arbuscular mycorrhizas.

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