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An update on nutrient transport processes in ectomycorrhizas

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Abstract

Nutrient transport, namely absorption from the soil solution as well as nutrient transfer from fungus to plant and carbon movement from plant to fungus are key features of mycorrhizal symbiosis. This review summarizes our current understanding of nutrient transport processes in ectomycorrhizal fungi and ectomycorrhizas. The identification of nutrient uptake mechanisms is a key issue in understanding nutrition of ectomycorrhizal plants. With the ongoing functional analysis of nutrient transporters, identified during sequencing of fungal and tree genomes, a picture of individual transport systems should be soon available, with their molecular functions assessed by functional characterization in, e.g., yeast mutant strains or *Xenopus* oocytes. Beyond the molecular function, systematic searches for knockout mutants will allow us to obtain a full understanding of the role of the individual transporter genes in the physiology of the symbionts. The mechanisms by which fungal and plant cells obtain, process and integrate information regarding nutrient levels in the external environment and the plant demand will be analyzed.

Introduction

The favourable effects of ectomycorrhizal (ECM) fungi on plant nutrition have traditionally been attributed to the quantitative effect of the extraradical mycelium on uptake of dissolved nutrients from the soil solution (Smith and Read, 1997). This interconnected network of hyphae (or specialized aggregates, i.e. rhizomorphs) forms a supracellular compartment for the transport of nutrients from sites of nutrient capture to sites of nutrient utilization and transfer. It has been estimated that the external mycelium made by far the greatest contribution to the overall potential absorbing surface area of pine seedlings inoculated with *Pisolithus tinctorius* or *Cenococcum geophilum* (Rousseau et al., 1994). Attention has been also paid to the utilization of organic nitrogen and phosphorus forms from more complex substrates (Perez-Moreno and Read, 2000; Smith and Read, 1997), and to the direct mobilization of nutrients from minerals (for review, see Landeweert et al., 2001). Although interactions between mycorrhizal fungi and other soil microbes will not be detailed in the present review, this key feature may have a substantial role on the nutrient economy of ectomycorrhizal trees, with respect to hydrolysis of complex organic compounds (Koide and Kabir, 2001) or organic acid excretion (Landeweert et al., 2001). Translocation through the hyphal network and further transfer of nutrients from fungus to host root has also been discussed in detail (Smith and Read, 1997), but the intimate anatomical connections between fungal and root cells presents considerable technical difficulties for unambiguous experimental investigations of nutrient transfer between fungus and host. Biochemical studies using detached mycorrhizal roots or mycelia revealed the kinetic complexity of

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nutrient transport. The interpretation of these studies was mainly hampered by the multiplicity of cell types in symbiotic tissues and the uncertainty of whether the different transport activities derived from one or more cell types (Javelle et al., 1999; Kreuzwieser et al., 2000; Van Tichelen and Colpaert, 2000). It is therefore essential to isolate individual carriers and to study their properties, function and regulation before relating them back to the whole plant/fungus level. This review will cover recent studies that have led us to consider the transport processes occurring in the ectomycorrhizal network as a key determinant in the nutrient economy of ectomycorrhizal plants. Recent papers have reviewed the actual knowledge in plant-fungus interactions on the basis of C and N availability as investigated on selected laboratory models (Buscot et al., 2000; Hampp et al., 1999; Nehls et al., 2001), and therefore this aspect will not be detailed in the present paper, except at the molecular level.

Mobilization, absorption, translocation and transfer of nutrients

Phosphorus

Although the total amount of P in the soil may be high, it is a very immobile and unavailable element for plant uptake because of its adsorption, its precipitation or its conversion to recalcitrant organic forms. It has been suggested that ECM fungi stimulate the uptake of P from poorly soluble sources in soil. Using mycorrhizal birch seedlings grown in observation chambers with the ECM fungus *Paxillus involutus*, losses of P from a range of different fermentation horizons could be measured (Perez-Moreno and Read, 2000). This ability to forage P from litter horizons was related to the capacity of the fungus to produce the necessary enzymes (Bending and Read, 1995). Wallander et al. (1997) showed that some ECM fungi were able to improve the uptake of apatite-P by ECM pine seedlings. However, weathering capacities clearly varied among ECM fungi and it has been recently found that apatite-weathering ability of ECM fungi were somehow related to their ability to produce oxalic acid (Wallander, 2000a).

Furthermore, uptake of P by mycorrhizal fungi is a key determinant of the improved P nutrition of ectomycorrhizal plants. Kinetics of net phosphate uptake by mycorrhizal *versus* non-mycorrhizal pine seedlings has been recently studied in details (Colpaert et al., 1999; Van Tichelen and Colpaert, 2000). The intact extraradical mycelium greatly increased the absorption surface area of the roots (Van Tichelen and Colpaert, 2000). *P. involutus* was found to be the most efficient mycobiont with regard to P influx, with a lower K_m value than the other pine-fungus associations tested in this study (Colpaert et al. 1999, Figure 1). Comparing the effects of arbuscular mycorrhizal (AM) and ectomycorrhizal fungi on P uptake by *Eucalyptus coccifera*, Jones et al. (1998) found that P inflow rates of ECM and AM seedlings were 3.8 times, and 2.0–2.7 times those of non-mycorrhizal seedlings. They also found that ECM and AM plants are more efficient than NM plants at acquiring P per unit of C allocated below-ground. Although numerous data clearly indicate that ECM fungi can improve P_i uptake, determination of P fluxes under various conditions also clearly demonstrate the heterogeneity of P uptake capacities among different mycobionts (Cairney and Smith, 1993). The identification of the P uptake mechanisms is therefore a key issue in understanding P nutrition of ectomycorrhizal plants.

In a recent experiment, Jentschke et al. (2001a) demonstrated that *P. involutus* hyphae were able to take up and translocate significant amounts of P to their P-deficient host plant and therefore strongly improved host P status that in turn stimulated seedling growth. There is increasing evidence that P translocation through hyphae occurs by active processes (Ashford, 1998; Timonen et al., 1996). A role of tubular vacuole system in longitudinal transport of P in *P. tinctorius* hyphae has been hypothesized (Cole et al., 1998; see Ashford and Alloway, 2002). Using X-ray spectroscopy, Bücking and Heyser (2000a) concluded that the translocation of P from fungus to host plant across the mycorrhizal interface is driven by P concentration in the cytoplasm of the Hartig net and by efflux rate into the interfacial apoplast. However, the detailed mechanism of P transfer at the symbiotic interface remains obscure.

Nitrogen

In forest soils, N is present either as NH_4^+ and/or as NO_3^- and organic compounds such as amino acids, peptides and proteins (Chalot and Brun, 1998; Marschner, 1995). The significance of mycorrhization for improved uptake of ammonium and organic N has been shown in several studies (Javelle et al., 1999; Plassard et al., 2000), whereas its influence on NO_3^-

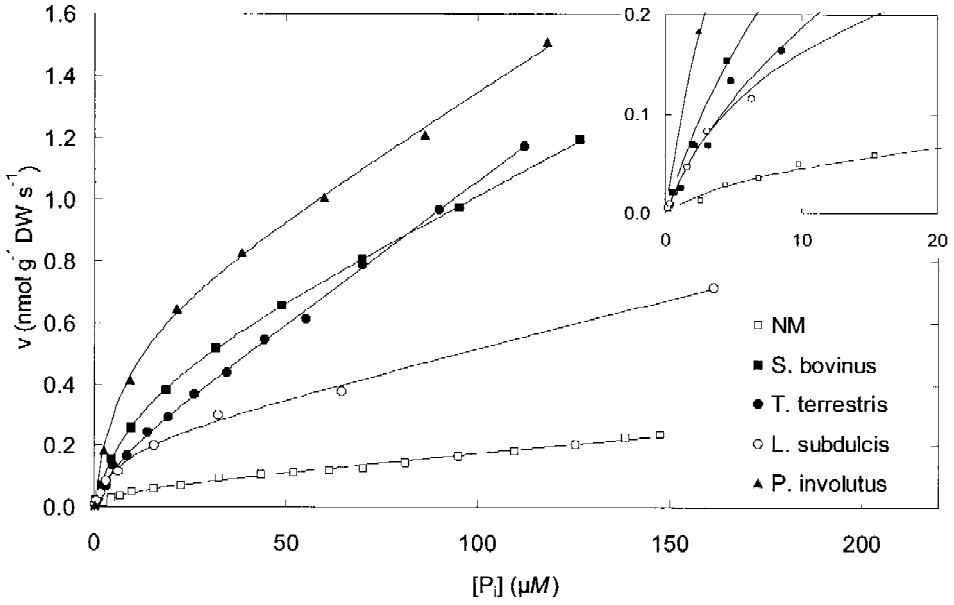

Figure 1. Michaelis–Menten plot showing the net P_i uptake in intact mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings as a function of solution P_i concentration. The inset figure shows the relationship at the lower P_i concentrations, most representative for soil solution P_i. Measurements were performed 9 weeks after inoculation and data points were fitted to a two-phase Michaelis–Menten equation. Data obtained with excised *Fagus sylvatica – Lactarius subdulcis* mycorrhiza are also plotted (Colpaert et al., 1999; with permission).

uptake is still a controversial issue (Kreuzwieser et al., 2000; Plassard et al., 2000).

Ammonium mobilization by hyphae from soil sources is directly linked to hyphae uptake capacities. The kinetics and energetics of ammonium/methylammonium transport in ectomycorrhizal fungi have been characterized (Javelle et al., 1999; Jongbloed et al., 1991). Comparing the ammonium uptake capacity of the two partners separately or in symbiosis, it was found that mycelia have much higher capacities for ammonium uptake than non-mycorrhizal roots and ectomycorrhizal fungi increase ammonium uptake capacities of their host roots (Javelle et al., 1999; Plassard et al., 1997). On the contrary, recent investigations indicated that mycorrhization leads to reduced rates of NO_3^- net uptake in *Laccaria laccata* mycorrhizal beech (Kreuzwieser et al., 1997, 2000). The authors attributed this effect to a reduced influx plus an enhanced efflux of NO_3^- as compared with non-mycorrhizal beech roots. The molecular mechanisms involved in inorganic N uptake will be further discussed in the last section.

A number of observations support the view that ectomycorrhizal fungi have the ability to degrade macromolecular nitrogen and furthermore to take up and assimilate the products of hydrolytic degradation (Chalot and Brun, 1998; Näsholm and Persson, 2001; Wallenda and Read, 1999). All of the major hydrolytic enzymes involved in mobilization of nitrogen from organic compounds have been detected in ericoid and some in ectomycorrhizal fungi (Chalot and Brun, 1998). Transport of amino acids was investigated in the mycorrhizal fungi *P. involutus* (Chalot et al., 1996) and *Amanita muscaria* (Nehls et al., 1999), which demonstrated their ability to take up a variety of amino acids. Recent data indicate that birch root colonization by *Paxillus involutus* increased glutamate uptake and that mycorrhiza formation induced a profound alteration of the metabolic fate of exogenously supplied glutamate (Blaudez et al., 2001). Plassard et al. (2000) further demonstrated that *Hebeloma cylindrosporum*/*Pinus pinaster* ectomycorrhizas had higher capacities for taking up glutamate compared with non-mycorrhizal pine roots. However, the critical question is to what extent this potential to use organic N occurs under natural conditions (Näsholm and Persson, 2001). The potential utilization of isotopic signatures for the determination of resource acquisition patterns by mycorrhizal plants has been discussed in detail (Evans, 2001; Hobbie et al., 1999). However, recent data indicates that this must be considered with caution, given the large isotopic fractionation patterns

during uptake and metabolism in mycorrhizal fungi (Emmerton et al., 2001).

Based on both ^{15}N labelling and mass balance data, it was demonstrated that hyphal ammonium acquisition contribute 45% to total plant N uptake under N deficiency (Jentschke et al., 2001b). In similar experiment using P deficient seedlings, they found that hyphal contribution to total plant N uptake amounted to 12% (Jentschke et al., 2001a). Disrupting the external mycelium from ectomycorrhizas greatly decreased [^{15}N]ammonium uptake by birch seedlings (Javelle et al., 1999). Therefore, external hyphae can be considered as the ammonium absorbing structure of ectomycorrhizal roots. These results confirmed the function of the extraradical mycelium in translocating N from sources to roots.

The physiological processes involved in the further transfer of N within the symbiotic tissues are still poorly understood. The exchange of nutrients between the fungus and the plant requires passage across the fungal plasmalemma, the interfacial matrix and plasmalemma of the plant. A number of hypotheses, which are derived from experiments on higher plants or bacteria, have been drawn in a previous review (Chalot and Brun, 1998). Indirect evidence from ^{15}N labeling experiments supports the view of amino acid transfer from the fungus to the host (Smith and Read, 1997). However a direct transfer of NH_4^+, as hypothesized for AM symbiosis (Bago et al., 2001), cannot be ruled out. The answer to whether amino acids or ammonium are preferentially transferred from the fungus to the host through the apoplast will probably arise from molecular approaches coupled with biochemical techniques. Direct analysis of apoplast fluid (Solomon and Oliver, 2001) together with tracer experiments for instance, will be necessary to elucidate transfer between fungal and root cells. Also detailed analysis of individual compounds using specific isotope analysis may be a key to further address these important questions (Evans, 2001).

Potassium, magnesium and calcium

Silicate minerals may provide calcium, magnesium and potassium, after chemical or biological dissolution (Landeweert et al., 2001). Potassium mobilization from apatite by mycorrhizal plants has been observed in long-term pot experiments (Wallander and Wickman, 1999; Wallander, 2000b). It was found that *Pinus sylvestris* seedlings colonized by *Suillus variegatus* enhanced K release from biotite as compared to non-mycorrhizal seedlings (Wallander and Wickman, 1999).

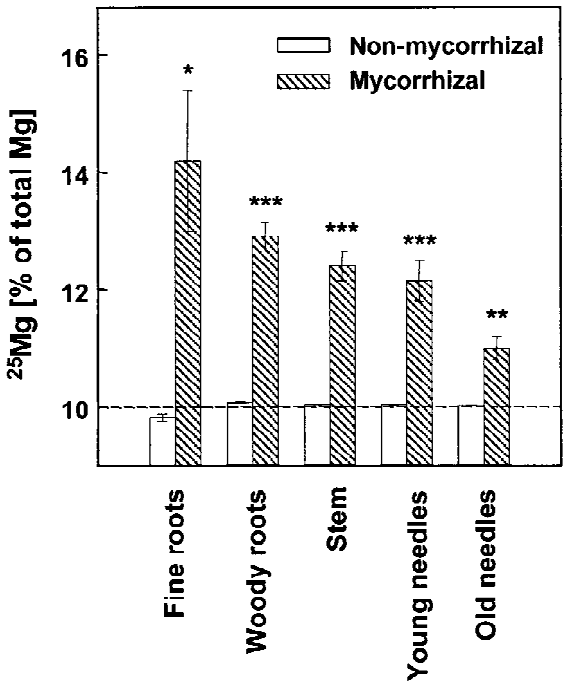

Figure 2. Concentration of the ^{25}Mg label (expressed as percentage ^{25}Mg of total Mg) in tissues of non-mycorrhizal and mycorrhizal Norway spruce seedlings after labeling the hyphal compartment in the culture system with ^{25}Mg for 6 weeks. Broken line indicates natural abundance of ^{25}Mg (10.0%). Values are mean values of 4 replicate pots. Small bars indicate standard error. Significance levels for differences between mycorrhizal and non-mycorrhizal seedlings: *, $P \leq 0.01$; **, $P \leq 0.001$; ***, $P \leq 0.0001$ (Jentschke et al., 2000, with permission).

Further translocation of K and Mg (Figure 2) through mycorrhizal hyphae has been recently examined (Jentschke et al., 2000; Jentschke et al., 2001a). Hyphal K and Mg acquisition by mycorrhizal spruce roots was significant and contributed to a minimum of 5–6% of total plant uptake (Jentschke et al., 2001a). These data demonstrate the potential role of the ectomycorrhizal mycelium in K and Mg acquisition.

Nutrient entry in root cells colonized by mycorrhizal fungi has been recently examined using isotopic tracers (^{44}Ca, ^{25}Mg and ^{41}K) and LAMMA (laser-microprobe-mass-analyser) (Kuhn et al., 2000). Based on entry kinetics and temperature dependence, it was concluded that Ca and Mg may enter the cortex via a free apoplastic path and that the endodermis is a major barrier for the further passage of these divalent cations into the xylem. For K, although kinetics were

not shown, the authors found a temperature dependence of K entry, which suggests that one component in K uptake mechanism may be carried out by an active process. This will be further discussed in the last section. However, depending on the fungal partner, variations in the intensity of apoplastic movement of nutrients could be observed (Bücking and Heyser, 2000b). For instance X-ray microanalyses indicate that in mycorrhizas of *Pinus sylvestris*/*Suillus bovinus* the apoplastic movement of cations across the fungal sheath is strongly reduced. In eucalypt mycorrhizas, the exodermal Casparian bands have a low permeability to apoplasmic tracers, suggesting that an isolated shared apoplasmic compartment exists at the interface between the partners, also not tightly sealed (Vesk et al., 2000).

Interdependence of nutrient translocation

P deficiency in spruce triggers hyphal foraging and translocation not only of P from P-rich sites, but also of N, K and Mg, which indicates potential co-translocation of a limiting element (P) with non-limiting nutrients (N, K, Mg) by *P. involutus* (Jentschke et al., 2001a). In this experiment, K translocation was of the same order of magnitude as P translocation whereas N translocation exceeded P translocation five-fold. Mg fluxes through hyphae were the lowest of all nutrient fluxes determined. In agreement with these studies, Cole et al. (1998), using X-ray mapping demonstrated that vacuoles in growing hyphae retain high levels of P and K.

Transport processes at the molecular level

Fungal transporters: a Hebeloma cylindrosporum *database*

Genomic and cDNA libraries have been prepared, and an EST (expressed sequence tags) resource of ca. 3000 sequences has been produced (Table 1). Preliminary analysis of the library reveals that ca. 60% of the tags correspond to genes that have counterparts in other organisms, and 50% of known function. Among them, 38 tags correspond to genes encoding carriers or channels that could play a role in nutrient uptake from the soil solution or from the host plant apoplast, or in nutrient secretion towards the host plant (Table 1). Of particular interest are several K^+ channels (homologous to Shaker/Shab channels identified in animals), one K^+ carrier (homologous to yeast TRK systems), 2 Pi transporters (homologous to yeast and *Glomus versiforme* Pi uptake systems), one NH_4^+ carrier, one peptide carrier, several hexose carriers, organic acid transporters, a large set of ATP binding cassette (ABC) transporters, or metal (Fe, Cu, Zn) transporters. Also, several enzymes that could play crucial roles in improving P (phosphatases) and N (aminotransferases, glutamine synthetase, glutamate synthase, urease, asparagine synthetase) nutrition have been identified.

Ammonium transporters

Ammonium transporters were first cloned from yeast (Mep1) and higher plant (Amt1) (Marini et al., 1994; Ninnemann et al., 1994). Heterologous expression of the yeast triple *mep*Δ mutant (Marini et al., 1997) has enabled the characterization of HcAmt2 and HcAmt3 from *H. cylindrosporum*, the first members of the ammonium transporter protein family (Amt) in basidiomycetes (Figure 3). HcAmt2 expressed in yeast had a higher affinity but a lower capacity than HcAmt3. Northern blot analyses in *H. cylindrosporum* showed that *HcAMT2* and *HcAMT3* were up-regulated upon nitrogen deprivation and down-regulated by ammonium (Javelle et al., 2001). A third member of this family showing a high homology to HcAmt2 was recently isolated by direct complementation from a cDNA library (Javelle, unpublished results). Results also indicate that the HcAmts could play a role in ammonium retention in *H. cylindrosporum*, as already demonstrated for the Mep proteins in *S. cerevisiae* (Marini et al., 1997). An immunological approach is currently being developed in our laboratory to elucidate the intriguing questions concerning the post-translational regulation of this protein family and its cytolocalisation in ectomycorrhizas.

Amino acid transporters

Amino acid transporters have been characterized physiologically in detail in animals, plants and yeasts (for review see Fischer et al., 1998; van Belle and André, 2001; Williams and Miller, 2001; Wipf et al., 2002). On the basis of physiological studies, the existence of a large number of transporters has been postulated differing in their substrate spectrum, in their transport mechanism, i.e. the ions used in co-transport, and in tissue specificity. Three transporter superfamilies are found in plants and yeast (the APC, amino acid-polyamine-choline transporter superfamily, the ATF, amino acid transporter superfamily, the

Table 1. Hebeloma cylindrosporum EST resource

Protein function prediction	Number of clones (Percentage of the matching ESTs)	Number of putative different proteins
Metabolism	367 (34,04)	172
Protein synthesis	256 (23,74)	131
Postranscriptional modification and proteolysis	215 (19,94)	76
Cytoskeleton	56 (5,19)	22
Signal transduction	61 (5,65)	48
Nuclear proteins	28 (2,59)	23
Transporters	38 (3,52)	29
Probable membrane proteins	28 (2,59)	19
Cell wall proteins	29 (2,69)	15
Unclassified	855	655

[a]Protein function prediction from ESTs was made with BlastX program (Altschul et al., 1990). Around 1000 sequences were absent from this table because they did not match with any protein of database (expect $> e^{-2}$). ESTs matching known proteins with BlastX expect $< e^{-15}$ have been classified into functional categories, ESTs with expect $> e^{-15}$ were placed into unclassified category together with ESTs which can not be classified into functional categories. The percentage of each individual functional class (calculated from the total number of classified proteins) is given in parenthesis. Third column indicates number of different accession numbers obtained in the first line of BlastX program.

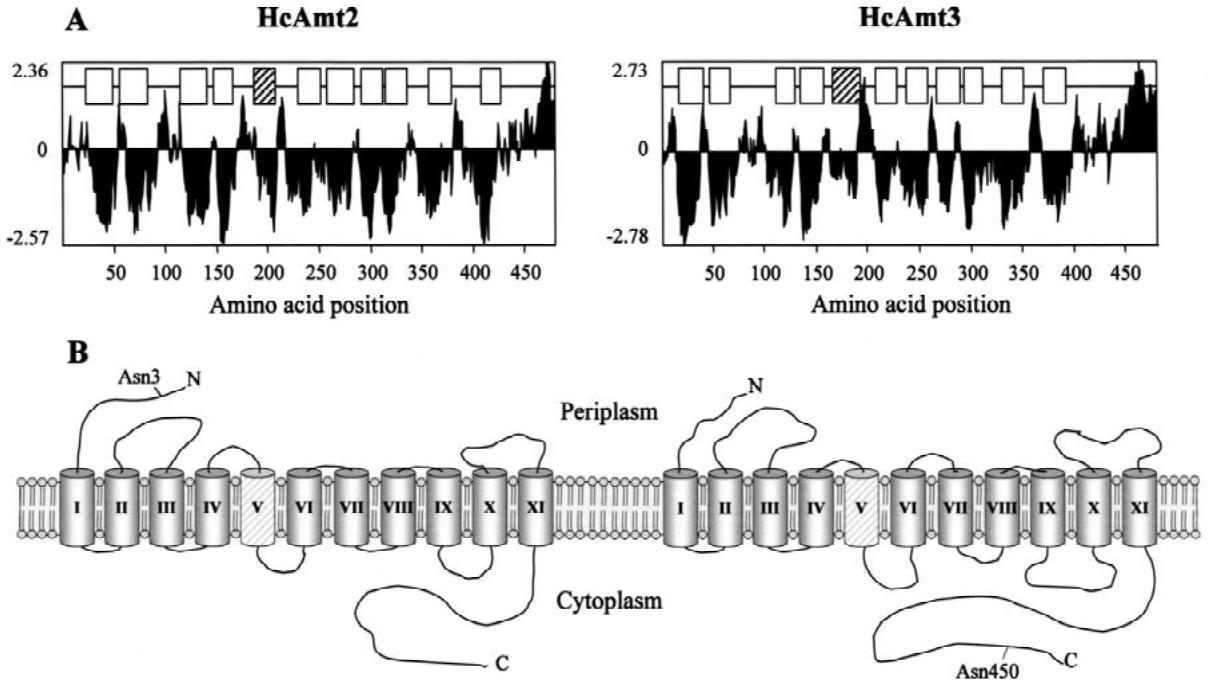

Figure 3. Topology of the ammonium transporters from *Hebeloma cylindrosporum*. (A) Hydropathy profile of the HcAmt proteins. Hydropathy analyses were performed using the Kyte and Doolittle algorithm. The upper boxes represent the relative positions of the membrane-spanning segments predicted by the algorithm TMpred, HMMTOP and TMHMM. The hatched boxes correspond to the ammonium-signature containing-TM. (B) Schematic model of the two HcAmt proteins. The predicted glycosylation sites (Asn3 for HcAmt2, Asn450 for HcAmt3) are indicated. The hatched boxes correspond to ammonium-signature containing-transmembranes (Javelle et al., 2001, with permission).

VGT, vesicular glutamate transporter family). Recently, two amino acid transporters from mycorrhizal fungi have been characterized. AmAap1 was identified from *A. muscaria* by screening a cDNA library (Nehls et al., 1999). HcBap1 was isolated from *H. cylindrosporum* by functional complementation of a yeast strain deficient in amino acid transporters (Wipf et al., unpublished results). Their expression in yeast revealed that they function as high affinity transporters. Two main functions can be expected for those genes, namely uptake of amino acid from the soil for nutrition, and the prevention of amino acid loss by hyphal leakage in the absence of a suitable N source at a low internal N status. However, given the variety of amino acid transporters described in animals, plants and yeasts, the characterization of many more fungal transporters is expected and needed.

Potassium transporters

Potassium transport has received much attention in plants and fungi (for review see Rodriguez-Navarro, 2000). It is clear that the EST resource described above now offers invaluable molecular tools for studying the molecular mechanisms of K transport in ectomycorrhizal fungi. For instance, regarding nutrient ion uptake and secretion towards the host plant, sequence analysis of the clones corresponding to the K^+ transport systems identified in the EST library provides the following working hypotheses. The K^+ carrier that displays similarities with the yeast TRK systems could be responsible for high affinity active K^+ uptake by peripheral hyphae from the soil solution. On the other hand, the K^+ channels displaying similarities with animal Shaker/Shab outward K^+ channels could play a role in K^+ secretion towards the host plant. Within these working hypotheses, a preliminary step will be to assess the molecular function of these transport systems via their expression in heterologous systems (e.g. functional complementation of yeast mutants, electrophysiological characterization in *Xenopus* oocytes).

Host tree transporters

Although a variety of ammonium, amino acid, peptide, phosphate and sugar transporters have been confirmed to be expressed in plant roots (Delrot et al., 2000; Fischer et al., 1998; von Wiren et al., 2000), data on tree roots is lacking and urgently needed. Current sequencing projects (i.e. poplar database, http://mycor.nancy.inra.fr/PoplarDB.html) may provide potential tools for detailed analysis of transporter genes in tree roots.

Tree & fungal hexose transporters

It is assumed that sucrose is delivered into the apoplast at the plant-fungus interface (Hartig net) and hydrolyzed via a plant-derived acid invertase (Nehls et al., 2001). The resulting hexoses are then taken up by fungal cells as well as by plant root cells (Nehls et al., 2000; Wright et al., 2000). A prerequisite for rapid uptake of monosaccharides is a membrane transport system. So far only one hexose transporter (AmMst1) has been identified from the ectomycorrhizal fungus *A. muscaria* (Nehls et al., 1998). The expression of AmMst1 in a *Saccharomyces cerevisiae* strain, unable to take up hexoses, demonstrated that AmMst1 was a functional monosaccharide transporter (Wiese et al., 2000). A *Picea abies* hexose transporter cDNA (*PaMST1*) that encodes an open reading frame of 513 amino acids was isolated by a RT-PCR based strategy (Nehls et al., 2000). PaMst1 is expressed in the hypocotyl and in roots of *P. abies* seedlings, but not in needles sampled at different developmental stages (cotyledons and young needles). In addition, two putative hexose transporter gene fragments were identified from birch by RT-PCR (Wright et al., 2000). While the expression of the hexose transporter gene of Norway spruce was only slightly (approx. 30%) reduced in mycorrhizas, the transcript level of both hexose transporter genes of birch was reduced by a factor of three. Even if a reduced transcript level does not mean a decreased transporter activity, and although the number of monosaccharide transporters expressed in plant roots is not yet clear, these results suggest that plants do not increase their hexose import capacity during symbiosis. In contrast, the expression of the fungal monosaccharide transporter gene increases significantly in mycorrhizas. Since, in addition, the transcript level of the fungal (*A. muscaria*) monosaccharide transporter gene is much higher than that encoding the *P. abies* hexose transporter (U. Nehls, unpublished), the fungus represents the major carbohydrate sink in infected fine roots. It could thus be assumed that the plant does not compete for hexose import at the plant-fungus interface, and that the fungal activity determines the sink strength for carbohydrates in mycorrhizas.

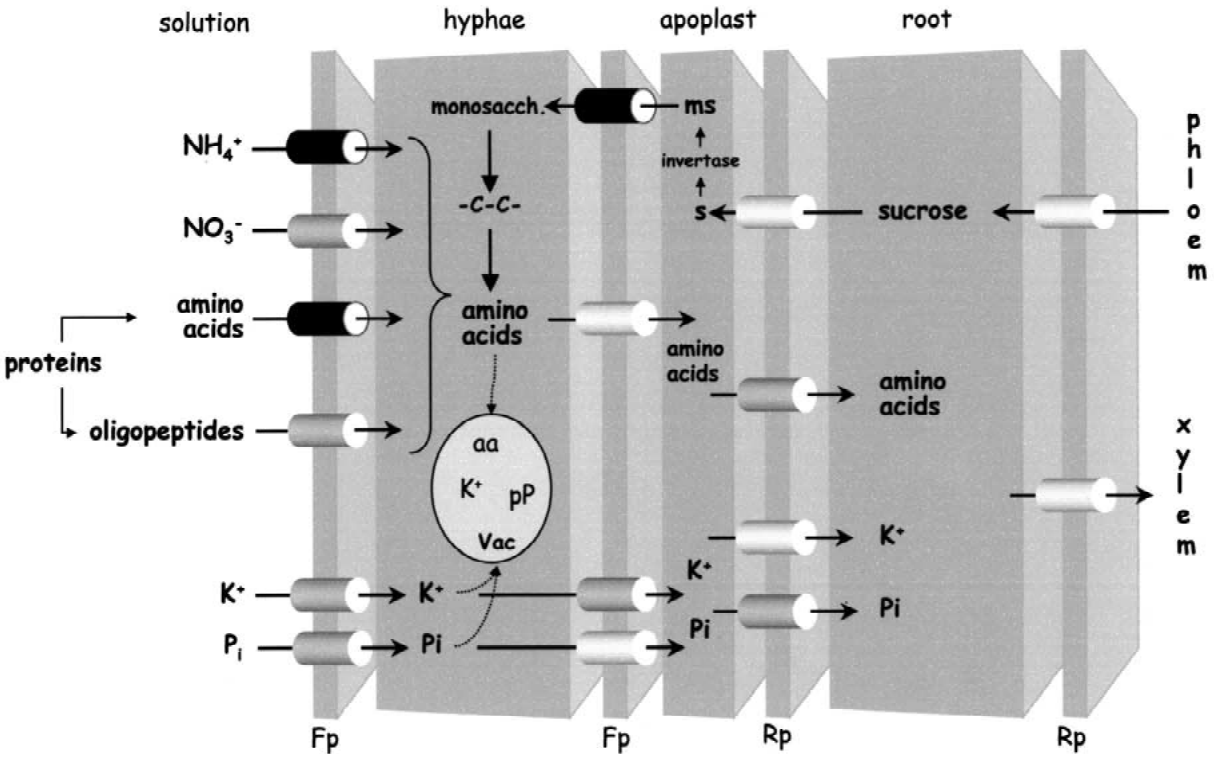

Figure 4. Summary of current understanding of transporters in ectomycorrhizal tissues. fp: fungal plasma membrane, rp: root plasma membrane, pP: polyphosphate. Black cylinder: one member of the transporter family has been fully characterized, i.e., functional complementation of a yeast deficient strain; grey cylinder: putative transporters (candidate genes available in i.e. EST resources); white cylinder: hypothetic transporters.

Transporters in ectomycorrhizal tissues: overview and perspectives

Our current understanding of transport processes in ectomycorrhizal tissues is summarized in Figure 4. Up to now, only 5 transporters from ectomycorrhizal fungi have been fully characterized, i.e., their function demonstrated by functional complementation of deficient yeasts (HcAmt2, HcAmt3, Javelle et al., 2001; HcBap1, Wipf et al., unpublished results; AmMst1, Nehls et al., 1998; AmAap1, Nehls et al., 1999). A number of other candidate genes from fungi or from host roots have been isolated either by degenerate PCR or from EST resources. However, the precise function and localization of these putative proteins in mycorrhizal tissues remain unknown. Nutrient fluxes from fungus to host largely depend upon coordination with nutrient release by the fungus into, and nutrient reuptake by the root cells from, the apoplastic interface. Unequivocal elucidation of these transport mechanisms will rely on innovative physiological and molecular approaches. Heterologous complementation of deficient yeasts with transporter genes and further expression analysis will probably help in understanding the uptake processes.

However, the most difficult mechanism to perceive will be the release of amino acids into the apoplast by the fungus. Studies indicate that amino acid release by ectomycorrhizal fungi indeed does occur (Plassard et al., 1996). Whether the mechanism involved, specifically applied to the symbiotic interface, and whether it concerns a limited range of amino acids remain intriguing questions. The lack of deficient yeasts in amino acid excretion greatly hampered the study of amino acid excretion in other organisms. Research in these directions however are in progress and should allow the characterization of amino acid exporters in mycorrhizal fungi in the near future.

Current research work on the model plant *Arabidopsis thaliana* clearly illustrates the usefulness of models allowing development of genetic, reverse genetic and transgenesis approaches (Zimmermann and Sentenac, 1999). For instance, expression of promoter-reporter gene constructs in transgenic fungi

as well as immunological-based approaches will allow us to localize the expression of these various transport systems in the fungus (peripheral hyphae, Hartig network) and to study the effects of nutrient deficiency on their expression. In a recent work, the expression and the cellular localization of a proline transporter was successfully studied in *Aspergillus nidulans* using GFP fusion protein (Tavoularis et al., 2001). It is exciting to consider the value of this approach in studies of plant-fungus interactions, especially since fluorescent protein labeling patterns can be extended to the ultrastructural level by immunolocalization with GFP antibodies (Gordon et al., 2000). However, use of this technology relies on the availability of high throughput transformation system for mycorrhizal fungi. In some of the experiments described in the present paper, *H. cylindrosporum* was chosen as a model of ectomycorrhizal fungus, since this species can be grown in vitro from spore to spore (Debaud and Gay, 1987) and can be transformed (Marmeisse et al., 1992), allowing genetic and reverse genetic approaches. More recently, a technique was developed for transforming mycorrhizal fungi, including *H. cylindrosporum,* based on *Agrobacterium tumefaciens* mediated T-DNA transfer (Pardo et al., 2002). *Agrobacterium tumefaciens* mediated transformation has been successfully applied to the wheat pathogen *Mycosphaerella graminicola* for the disruption of an ABC transporter, generated disruptants with high efficiency (Zwiers and De Waard, 2001).

Ultimately, the combination of biochemical and molecular approaches will provide a platform for discovering how release and transport events at the apoplastic interface are coordinated and how controls either by the fungus or by the host are exerted.

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