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The ectomycorrhizal symbiosis: genetics and development

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Abstract

Ectomycorrhiza represents a symbiotic structure made between tree roots and filamentous hyphae. This new organ results from a favourable interaction between plant and microbes, taking place in the soil at the vicinity of the root. Diversity is extremely important in the rhizosphere, with large numbers of bacterial, fungal, nematode and invertebrate species. Therefore, partners of the mycorrhiza have to recognize each other and they do it by using diffusible rhizospheric molecules. This recognition leads to cellular interactions between root and fungal cells, driven by changes in gene and protein expression. The aim of this review is to describe the cellular, genetic and molecular events leading to the formation of the ectomycorrhizal tissues with an emphasis on gene expression and cell-to-cell communication.

Introduction: Communication is harmony

Living in symbiosis sounds like each partner being in harmony. However, communication in a couple is essential for a stable and enriching relationship. This seems to be the case for the mutualistic ectomycorrhizal symbiosis, a well described association which allow trees to adapt and support adverse conditions. The key to this equilibrium is probably due to a finely tuned balance between (i) genetic determinism of the ability to form a symbiosis, and (ii) environmental conditions which may enhance or repress the establishment of the relationship. Cell sensing of the environnement and cell-to-cell communications within the ectomycorrhizal tissues likely play a major role in this interchange. Any breakdown of the relationship leads to a disruption of the symbiosis. This may happen in winter for temperate climates, or during root and hyphal senescence. This may also have happened several times through evolution, since some mycorrhizal species appear to have reverted to a free-living condition (Hibbett et al., 2000).

Ectomycorrhizas are abundant mainly under temperate and boreal climates, between tree species (Angiosperms and Gymnosperms) and soil-borne filamentous fungi (ascomycetes and basidiomycetes essentially) (Smith and Read, 1997). Ectomycorrhizal mycelium growing into the substrate connects to host roots and forms a mutualistic symbiosis where partners are reciprocally benefited. Photosynthetic carbohydrates are translocated from the plant to the heterotrophic fungus which needs this rich carbon source to develop. Hyphae growing in the substrate explore the soil and absorb minerals and water which are in part tranferred to the root (Smith and Read, 1997). This exchange of metabolites is essential for the persistence of both tree and mycelium, mainly in disturbed environmental conditions. This symbiosis plays an essential role in the stability of forest ecosystems, and fulfills a role in the protection of root systems from pathogenic attacks, and from adverse abiotic soil conditions like water-stress (Smith and Read, 1997).

To allow or maintain communication and integrity in symbiotic tissues, fungal and root cells have probably developed sensing molecules (cell-anchor receptors and mobile signal-ligands similar to adhesins) (Corrêa et al., 1996) to (i) rapidly adapt to changes in their close cellular environment and (ii) send to the nucleus intra-cellular signals through transduction pathways. These cellular and molecular adaptations

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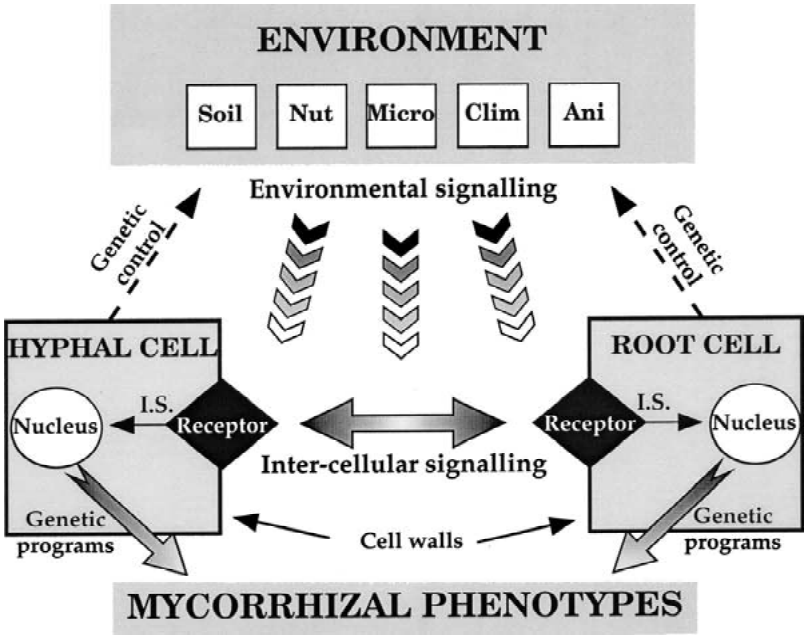

Figure 1. Inter- and intra-cellular communications between fungal hyphae and root cells in the ectomycorrhiza symbiosis. Changes in environmental conditions may produce signals sensed by both partner cells which probably transduce this information to their nuclei and provoke modifications in gene expression and consequently in phenotypes. I.S. – Intra-cellular signalling. Nut – nutrients. Micro – microorganisms. Clim – climatic factors. Ani – animals (human included).

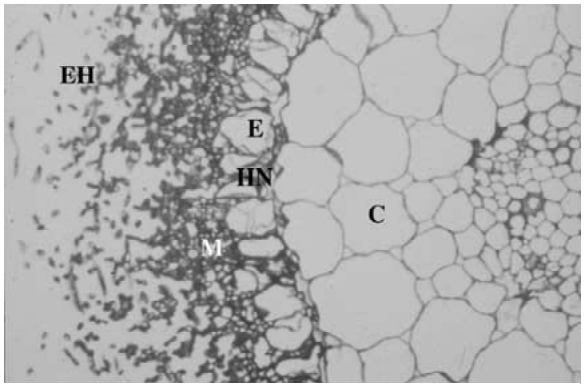

Figure 2. Cross-section of *Eucalyptus globulus* - *Pisolithus* ectomycorrhiza showing the mantle (M) ensheathing the epidermal root cells (E), the Hartig net (HN) made of fungal hyphae penetrating between radially elongated epidermal cells. EH – extra-matrical hyphae. Photographs by E. Carnero-Diaz, D. Tagu and F. Martin. Magnification: 700×.

are dependent on cell wall structure, organization and plasticity since fungal and root cells communicate through their apoplasts. In this review – after describing the ectomycorrhizal system – we present the actual knowledge on signalling and cell surfaces and their role in ectomycorrhiza formation (Figure 1).

Ectomycorrhizas: A sustainable interaction ?

Structure and function

Despite the large number of plant and fungal species able to form ectomycorrhizas, the general organization of the symbiotic tissues is conserved, from which variations in color, density, size and forms could appear. Ectomycorrhiza is defined as a modified lateral root. Colonizer fungal hyphae surround the root and form a sheath which isolates the root surface from its substrate (Figure 2). This ectomycorrhizal mantle is connected to a highly extended network of hyphae exploring the soil. Fruitbodies and fungal mats are formed from the differentiation of these extra-radical hyphae. Hyphae in the mantle are also connected to the root by an intra-radicular structure called the Hartig net, which is the structural and functional interface between fungal and root cells. It is made by the colonization of epidermal or cortical root cells by hyphae. In the ectomycorrhiza, the contact remains exclusively through the apoplastic compartments of both partners.

This basal organization corresponds to compartmentalization of functions. Extra-radical hyphae are responsible for mineral nutrition and water uptake. Minerals and water are taken up by hyphal tips and

their distribution within the other part of the mycelium – including symbiotic hyphae – is driven by long distance trafficking of solutes involving vesicles, vacuoles and tubules (Cole et al., 1998). Extra-radical hyphae can aggregate to form multi-hyphae structures (rhizomorphs, cords) particularly adapted to long distance transport of solutes (Cairney, 1992). The ectomycorrhizal mantle is a storage compartment (Kottke et al., 1995), a buffer zone between the 'sampling' hyphae (extra-radical net) and the 'exchanging' hyphae (intra-radical net). The Hartig net is the privileged place for metabolite exchange between root cells and fungal cells. It is made of specialized hyphae presenting extended and invaginated plasma membranes that amplify the contact zone with root cells. Cell walls are altered in composition and structure compared to free living conditions (Bonfante, 2001) and their plasticity reflects their role in intra- and inter-cellular communication.

The final symbiotic tissue results from a highly physical integration of root and fungal cells. Such a cellular 'understanding' between the two partners is driven and finely regulated in time and space by interactions between plant and fungal genetic programs involved in sensing the environment, cell-to-cell communication, and constructing new cellular structures.

Genetic versus *environment*

Some ectomycorrhizal fungal species are specific to certain host plants while others are not (Kropp and Anderson, 1994) and several genetic studies indicate that the ability to form ectomycorrhiza is a heritable trait and probably polygenically controlled in fungi (e.g. Debaud et al., 1988; Lamhamedi et al., 1990). Establishment of genetic maps for ectomycorrhizal fungi are urgently needed for further understandings of the fungal genetic components of this symbiosis (Doudrick et al., 1995). Genetic maps for ectomycorrhizal trees have been available for several years, but very little data on the inheritance of the ability to form ectomycorrhiza is described. In recent studies, we have characterized the variability to form ectomycorrhizas generated by an interspecific cross in poplar, comparing the full-sib progeny to the parental genotypes. In a pilot experiment (comparing 18 progenies), we have demonstrated that 3 months after controlled inoculation with *Laccaria bicolor*, the ability to form ectomycorrhiza was genetically determined as a dominant and male inherited trait (Tagu et al., 2001b). A broad sense heritability value of 0.49 was proposed, indicating a moderate but still important genetic control of the trait. In a larger scale experiment performed on 150 progenies of the same poplar family (Tagu et al., unpublished), a QTL (quantitative trait loci) was localized on the genetic map of the parental genotype *Populus trichocarpa*. This QTL is localized to a linkage group already characterized as involved in plant-microbe-interactions between poplar leaves and the pathogenic fungus *Melampsora larici-populina* responsible for rust infection. This is the first genetic evidence that the ectomycorrhizal trait is associated to genetic bases which might be partly common to mutualistic and pathogenic fungi.

Development

Morphological and ultrastructural changes

The formation of an ectomycorrhiza is a process which follows a timely regulated succession of morphological modifications during root colonization by hyphae. In the pre-infection step, hyphae sense the presence of a host root. Colonizer hyphae can be connected to a germinated spore and have low reserves of metabolites and energy. Colonizer hyphae can also be connected to fungal cells already involved in mycorrhizas. Colonization of new root tips from a mycorrhized short root occurs before the emergence of the new roots (Tranvan et al., 2000). Cell-to-cell communication processes occurring during mycorrhiza formation from these two types of hyphae might be different since the nature and the concentration of signalling molecules may differ.

After the pre-infection step, hyphae reach the root surface. This rapidly induces large modifications in the morphology of the fungal cells. Hyphae branch and form several new tips. Fungal cells in contact with the root cell wall swell and adhere firmly to the root. Hyphal branching is probably triggered by root exudates (see below), whereas swelling might be stimulated by topographic sensing of the root surface. Colonization of the root surface is accompanied by the inhibition of pre-existing root hairs. Fungal cells excrete a fibrillar material towards the root surface which probably plays a role in anchoring hyphae. A similar fibrillar material is also deposited between two adjacent hyphae forming the mantle. Mantle formation induces important changes in extra-matrical material and involves appearance of new interfaces between fungal and root cells (Bonfante, 2001). Hyphae forming the Hartig net progress within the apoplastic compartment of the root: Cell walls and plasma membranes are highy in-

vaginated and cells become multinucleated and rich in mitochondria and endoplasmic reticulum, indicating a high metabolic activity within these cells. During progression and penetration of hyphae, colonized root cells of some angiosperm species change their orientation of growth and these modifications are probably correlated to cytoskeleton changes (Carnero Diaz et al., 1996; Gorfer et al., 2001; Niini et al., 1996; Tarkka et al., 2000). Root apoplast is thus drastically altered by the fungal ingress. The majority of these morphological modifications are accompanied by changes in gene expression and protein accumulation.

Changes in transcript and protein patterns

It has been known for more than 10 years that the formation of ectomycorrhizas is correlated with changes in polypeptide accumulation and synthesis in both partners (e.g. Hilbert et al., 1991; Simoneau et al., 1993; Tarkka et al., 1998). Depending on the plant or fungal species, and on the experimental system, the proportion of Symbiosis-Regulated (SR) proteins varies. Only a very few of these proteins have been characterized and their functions are still unknown (Martin et al., 1999; Tagu and Martin 1996).

Analyses of transcript populations accumulated in ectomycorrhizas allowed the identification of SR-genes (e.g. Kim et al., 1999; Tagu et al., 1993). However, the development of new approaches such as transcript profiling and differential hybridization of cDNA arrays allowed the recent characterization of the major plant and fungal genetic programs regulated by the formation of ectomycorrhizas. From the initial work developed on *Eucalyptus* – *Pisolithus* ectomycorrhizal association, it has been shown that more than 15% of the transcripts correspond to SR-genes, but no ectomycorrhiza-specific genes were detected in this study. This suggests that the contact between *Eucalyptus* and *Pisolithus* does not trigger one specific genetic program, but rather stimulates or represses pre-existing root and fungal genetic programs (Voiblet et al., 2001). Nevertheless, Voiblet et al. (2001) demonstrated that root defense- or stress-reactions are enhanced by hyphae colonization; fungal cell wall proteins are more abundant in regions in contact with the root cells, and intra-cellular signal transduction pathways are prevalent both in *Eucalyptus* roots and *Pisolithus* hyphae during the interaction. These data clearly suggest that the most reactive root and fungal cell responses concern (i) cell-to-cell communication and (ii) cell wall plasticity.

Signalling: How to pass the border

Inter-cellular signalling

Diversity is extremely important in the rhizosphere, with billions of bacterial, fungal, nematode and invertebrate species. Therefore, partners of the mycorrhiza have to recognize each other and they do it by using diffusible rhizospheric molecules. In the case of colonizer hyphae originating from spore germination, sensing the presence of a root system might be done by sensing modifications in nutrient components of the rhizosphere compared to bulk soil. Plant roots absorb nitrogen at their vicinity, creating a depleted zone in nitrogen, and excrete sugars, locally enriching the rhizosphere in carbohydrates. This N depletion and C enrichment could serve as trophic signals by soil-borne fungi for the presence of a root system, as already demonstrated for the foliar pathogenic fungus *Cladiosporium fulvum* (Pérez–García et al., 2001). However, these trophic changes are probably not specific, and could be used as well by saprotrophic, pathogenic or symbiotic fungi to recognize the presence of a living plant. Root and fungal exudates are probably the source of more specific signals, even though they have not been characterized in ectomycorrhiza yet. Root exudates are known to stimulate spore germination and hyphal growth of several ectomycorrhizal fungi (reviewed in Duplessis et al., 2001b). Flavonoids – which are abundant molecules of the secondary metabolism excreted by the root exudates – are involved in numerous plant – microbe interactions (Broughton et al., 2000; Paiva, 2000). Recently, Lagrange et al. (2001) identified a flavonol from *Eucalyptus* root exudates – the rutin – which stimulated growth of *Pisolithus* hyphae at very low concentrations (1 pM). The action of rutin was not specific to *Pisolithus* or even to ectomycorrhizal fungi. Zeatin – a plant cytokinin – was detected in eucalypt root exudates and was able to regulate *in vitro* branching angles of *Pisolithus* hyphae (Lagrande and Lapeyrie, 2001). Thus, rutin and zeatin play complementary roles in the signalling towards *Pisolithus* hyphae. More systematic screening of active root exudates molecules are needed for their better qualitative and quantitative characterization.

Fungal hyphae also excrete rhizospheric signals. It has been known for a long time that ectomycorrhizal species synthesize and excrete auxins in the substrate (reviewed by Barker and Tagu, 2000). Auxins serve as very potent morphogenetic signals towards root systems. At low concentrations, they increase root growth

and stimulate the formation of new meristems and lateral roots. These lateral roots are new targets for colonization by ectomycorrhizal hyphae. It has been clearly demonstrated that mutant fungal hyphae which hyper-produced auxins formed 3 – 5 times more mycorrhizas than a wild type mycelium (Gay et al., 1994). The use of auxin transport inhibitors indicated that ectomycorrhizal morphogenetic effects driven by auxins were dependent on the existence of concentration gradients (Kaska et al., 1999; Karabaghli–Degron et al., 1998). Once the fungal hyphae have surrounded the lateral root, subsequent division of root meristematic cells is blocked: It is hypothesized that root cells completely ensheated by ectomycorrhizal hyphae perceive a high concentration of auxins, which inhibits cell division (Barker and Tagu, 2000). These hormonal gradients are probably regulated by pools of conjugated auxins since the active auxins are known to be very unstable. Béguiristain et al. (1995) have identified an abundant indolic compound excreted by *Pisolithus* hyphae. This molecule – hypaphorine – is a tryptophane betaine and is highly accumulated in *Pisolithus* hyphal cells in contact with *Eucalyptus* roots. Hypaphorine is able to stimulate the expression of auxin-regulated genes in *Eucalyptus* and it was hypothesized that hypaphorine might act as an auxin-like compound (Nehls et al., 1998). However, recent data clearly indicate that hypaphorine is not an auxin-like molecule but, more surprisingly, counteracts auxin action, for example, on tap root elongation or root hair elongation (Ditengou and Lapeyrie, 2000; Ditengou et al., 2000). It is probable that hypaphorine competes with auxins for putative auxin-binding proteins and receptors (Kawano et al., 2001).

Intra-cellular signalling

Once signals have been released and sensed at the periphery of living cells, the information has to be transmitted to the cell nucleus to prevent or induce expression of specific genes responsible for the phenotype linked to the external signal. This transmission is performed by a cascade of biochemical regulations, called the transduction pathways. Nearly nothing is known about transduction pathways in ectomycorrhizas. Nevertheless, they are probably fundamental for ectomycorrhiza formation since in the case of pathogenic fungi, disruption of signal transduction genes often leads to the loss of pathogenicity (e. g. Mitchell and Dean, 1995; Regenfelder et al., 1997). Voiblet et al., (2001) demonstrated that genes involved in transduction pathways are among the most abundant (13% of the mRNAs) in ectomycorrhizal tissues. They correspond to sub-units of G-proteins, protein kinases and calcium binding proteins. A fungal α subunit of a G-protein was particularly stimulated in *Eucalyptus* - *Pisolithus* ectomycorrhizas, as well as a fungal Ras protein and a calmodulin gene from eucalyptus. A *ras* cDNA has also been described in the ectomycorrhizal fungus *Laccaria bicolor* and may be involved in the control of cell growth and proliferation of hyphae (Sundaram et al., 2001). Intracellular signalling in plant cells is also induced by fungal elicitors and triggered by G-proteins (Hebe et al., 1999). In turn, these plant G-proteins trigger efflux of anions and cations, extracellular alkalinization, phosphorylation or desphophorylation of unknown target proteins and finally, synthesis of H_2O_2 (Salzer and Boller, 2000).

Cell wall: A porous barrier

Cell walls and extra-cellular matrices must play several pivotal roles in mycorrhiza formation. They mainly control growth and cell extension of plant and fungal cells. They are the first cellular structures in the contact between the two partners: Apoplastic spaces are the obligate passage for signalling molecules and metabolites. Cell walls are connected to plasma membranes where receptors are anchored, and cell walls are responsible – together with the cytoskeleton – for cell structure and integrity. Mycorrhiza formation is accompanied by drastic changes in composition and structure of plant and fungal cell walls and extra-cellular matrices. The most demonstrative modification is the creation of new interfaces between fungal and root cells, where cell walls and plasma membranes are juxtaposed (Bonfante, 2001).

Chitin and ß-1,3 glucans of fungal origin are the main components of these interfaces (Bonfante, 2001; Martin et al., 1999) and their organization may change during ectomycorrhiza formation. The outer layer of fungal cell walls is connected to a fibrillar material which strongly reacts to a labelling specific to polysaccharides. These fungal fimbriae are secreted towards the root surface in the early stages of ectomycorrhiza development (Lei et al., 1990). The distribution of cystein rich components in the cell walls is completely changed between hyphae from the outer and the inner mantle (Paris et al., 1993). Biochemical and molecular analysis of *Pisolithus* – *Eucalyptus* ectomycorrhizas identified cell wall proteins regulated during the sym-

biosis (Martin et al., 1999; Tagu and Martin, 1996). These are cell wall mannoproteins of unknown structure and function (Tagu and Martin, 1996), alanin-rich acid polypeptides (the so-called SRAP-32 for Symbiosis Regulated Acidic Polypeptides of 32 kDa) and the hydrophobins. *Pisolithus* cell wall mannoproteins gp95 and gp72 are down regulated during the interactions with *Eucalyptus* roots. Concommitantly, SRAP-32 are highly synthesized.

SRAP-32 were first detected in two dimensionnal electrophoresis and are composed of at least 9 polypeptides differing by their apparent pI (from 4.5 to 5.5) and molecular weights (from 30 to 32 kDa). They correspond to a multigene family (Table 1). They are located in the cell walls of *Pisolithus* hyphae and are highly accumulated at fungal/root interfaces, and in penetrating hyphae forming the Hartig net (Laurent et al., 1999). Their sequences are unique to *Pisolithus* and no similarities to other proteins were found. Some of the SRAP-32 polypeptides have a signal peptide and may correspond to cell wall isoforms. Other SRAP-32 have no signal peptide and may correspond to cytosolic or vacuolar forms (Sorin and Tagu, unpublished) or secreted forms by an unknown mechanism similar to fungal galectins (Boulianne et al., 2000). Some members of the SRAP-32 family have an Arg-Gly-Asp (RGD) motif, found in many animal and microbial adhesins. This adhesion motif is known to be implicated in interactions with integrin receptors (Hynes, 1992). The role of the RGD motif found in SRAP-32 in adhesion is still speculative. It has been demonstrated that RGD motifs might also be involved in adhesion of fungal pathogens to host plasma membranes or cell walls by regulating the expression of plant defense responses during fungal penetration (Kiba et al., 1998; Mellersh and Heath, 2001). RNAs of SRAP-32 are among the most abundant in symbiotic hyphae. These genes are highly regulated during the formation of the mycorrhiza, mainly when mantle is formed and when Hartig net progresses (Martin et al., 1999). Altogether, these observations suggest that SRAP-32 could be involved in cell wall composition of hyphae during ectomycorrhiza formation.

Hydrophobins are cell wall proteins that have been well described in numerous filamentous fungi (Kershaw and Talbot, 1998). Their roles have been well studied in the saprotrophic fungus *Schizophyllum commune* for which it has been demonstrated that hydrophobins are very potent surfactant proteins (Wösten et al., 1999). Hydrophobins are able to self-assemble at any hydrophilic/hydrophobic interface. When hyphae are growing into the air, they are lined with a layer of hydrophobins: They direct their hydrophobic domains towards the air, thus rendering the surface of the hyphae very hydrophobic. This hydrophobin layer can also be involved in colonization by hyphae of any other hydrophobic surfaces like insect or plant cuticles, wood fragments or other hyphae (Wessels, 1997). Hydrophobin cDNAs have been found in *Pisolithus – Eucalyptus* ectomycorrhizas as 4 different sequences (Duplessis et al., 2001a; Tagu et al., 1996; Voiblet et al., 2001) (Table 1). The HYDPt-1 polypeptide has been localized at the surface of fungal cell walls (Tagu et al., 2001a). The HYDPt-1 polypeptide is able *in vitro* to decrease water surface tension (Bilewicz et al., 2001). *Pisolithus* hydrophobin mRNAs are – together with *Srap-32* transcripts – among the most abundant messengers in *Pisolithus* ectomycorrhiza and are strongly up-regulated in the early stages of symbiosis formation. They are probably involved in root colonization by hyphae and/or in agregation of hyphae during early mantle formation. Furthermore, the hydrophobicity of *Pisolithus* hyphae may reflect the importance of hydrophobins in allowing symbiotic hyphae to develop in air pockets often associated to *Pisolithus* ectomycorrhizas (Vesk et al., 2000). However, their precise roles are still ambigous and only targeted gene disruption experiments should provide further insights into these protein functions.

Both *Srap-32* and *HydPt* genes are highly up-regulated by ectomycorrhiza formation. However, the stimuli responsible for the up-regulation are not known. As suggested in Figure 1, trophic conditions could be one of this triggering. But in our conditions, no up-regulation of SRAP-32 and hydrophobin genes have been observed in different concentrations of N and C sources (Duplessis et al., 2001a, Sorin and Tagu, unpublished). The effect of root exudates have also been tested, but no regulation of these genes were observed upon contact of *Pisolithus* hyphae with eucalypt root exudates (Duplessis, Lagrange, Lapeyrie and Martin, unpublished). Thus, these highly abundant fungal cell wall proteins are probably regulated by other biotic or abiotic factors, or may represent co-regulated genes responsive to intrinsic developmental signals during ectomycorrhiza formation.

Conclusions

Ectomycorrhizal symbiosis is a complex interaction between two eucaryotic species. Despite the recent

Table 1. Pisolithus cell wall proteins regulated during ectomycorrhiza formation

	Number of ESTs[a]	Gene copies per haploid genome	Sub-classes[b]	Sequence characteristics	Properties	Cellular localization	mRNA accumulation
Hydrophobins	25	■ At least 4.	4	■ 100 amino acids in average. ■ 8 conserved cysteins. ■ Signal peptide. ■ Neutral pI.	■ Partial purification of the polypeptides. ■ Recombinant protein in *E. coli*. ■ Self-assembly of monomers. ■ Reduce water surface tension[c].	■ Cell wall located at the surface of saprotrophic and symbiotic hyphae[c].	■ Up-regulated (x 7) by mycorrhiza formation. ■ No regulation by N or C source availability[c]. ■ No regulation by root exudates[d].
Srap 32	27	■ At least 3.	3	■ 250 amino acids in average. ■ Presence of Arg-Gly-Asp motif for one sub-class. ■ Signal peptide for one sub-class. ■ Rich in Ala or Leu or Ser. ■ Acid pI (< 5,0).	■ Identification and characterization by 2D-PAGE[c].	■ Cell wall located at the surface of saprotrophic and symbiotic hyphae[c].	■Up-regulated (x 5) by mycorrhiza formation. ■ No regulation by N or C source availability[d]. ■ No regulation by root exudates[d].

[a] From 850 *E. globulus – P. tinctorius* ectomycorrhizas ESTs (Voiblet et al., 2001).
[b] A sub-class is defined when amino acid sequences diverge from more than 50% of similarity.
[c] Tagu et al. (1996); Laurent et al. (1999); Bilewicz et al. (2001); Duplessis et al. (2001a); Tagu et al. (2001a).
[d] Unpublished data.

development of cellular, genetic and molecular studies, our knowledge is still sparse since only very few signalling molecules, genes and proteins have been identified. A comprehensive view of cell-to-cell communications taking place in ectomycorrhizas requires further identification of nearly all the components involved in these processes. New technical approaches are now available whose aims are to make exhaustive lists of molecules in a given tissue. This may lead to indices of genes (genome sequencing) (International Human Genome Sequencing Consortium, 2001), of mRNA (transcriptome) (Velculescu et al., 1999), of polypeptides (proteome) (Pandey and Mann, 2000) or metabolites (metabolome) (Raamsdonk et al., 2001). These approaches, if applied to ectomycorrhizal partners, will represent departure points for further examination of the roles of these putative signalling molecules, SR-genes or SR-proteins. Gene disruption analyses should help in deciphering the function of plant or fungal SR-genes in the symbiosis. Generation of fungal and plant mutants, by gene tagging, mutated in the ectomycorrhizal phenotype would be very useful to meet this aim. For the first time, these comprehensive analyses seem possible within the next 10 years and will hopefully give a near complete image of the functioning of an ectomycorrhiza.

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