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REVIEW

Building a mycorrhizal cell: How to reach compatibility between plants and arbuscular mycorrhizal fungi

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Abstract

Arbuscular mycorrhizal fungi occur throughout the majority of ecosystems supporting host plant nutrition. Recent findings describe the accommodation of the fungus by the root cell as a crucial step for compatibility between the partners. We discuss here the novel aspects of cellular plant-fungus interactions, with a particular attention to the interface compartment, the unique apoplastic space hosting intracellular fungal structures. The main features of arbuscular mycorrhizal colonization are examined and recent information in the field of plant and fungal cell responses during the establishment of the symbiosis is discussed. Differences between the colonization of root epidermal and cortical tissues are discussed, highlighting the growing interest in the role of epidermal cells during the first and decisive steps of the symbiosis. New approaches such as root organ cultures, *in vivo* observations, GFP tagging and mutant plant analysis are commented on and information from these is compared with that gained from more traditional methods. In particular, the use of plant mutants is depicted as a powerful tool for dissecting and understanding the genetic and cellular aspects of plant/fungus compatibility. Finally, perspectives in this field are outlined through the application of these approaches to the currently unanswered questions.

Keywords: *Arbuscular mycorrhiza, cell responses, interface, symbiont accommodation*

Introduction

Arbuscular mycorrhizas (AM) appeared on earth at least 400 My ago, in the early Devonian (Remy et al. 1994). Since then, AM fungi, all belonging to the Phylum Glomeromycota (Schüßler et al. 2001), have spread throughout the majority of ecosystems and developed obligate symbiotic interactions with about 80% of land plant species (Van der Heijden & Sanders 2002) that provide them with organic carbon (Bago et al. 2000). The success of arbuscular mycorrhizas in evolution is mainly due to the central role that AM fungi play in the capture of nutrients from the soil and in their transfer to the host plant (Harrison et al. 2002, Govindarajulu et al. 2005). As a direct consequence, they are determinants of plant biodiversity, ecosystem variability and productivity of plant communities (Van der Heijden et al. 1998). Additionally, AM fungi interact with different classes of microorganisms in the rhizosphere, influencing this ecosystem to such an extent that a new term 'mycorrhizosphere' has been coined (Martin et al. 2000). Many recent excellent reviews have discussed the molecular and cellular aspects of AM as well as their genetics (Gianinazzi-Pearson & Brechenmacher 2004, Parniske 2004, Karandashov & Bucher 2005, Hause & Fester 2005, Harrison 2005). Here we discuss recent findings describing how the accommodation of the fungus by the root cell, including the formation of the interface, is a crucial step for the compatibility between the partners and for the construction of an 'arbuscular mycorrhizal cell'.

The interface: A key word for AM interactions

AM fungi can be described as a living interface located between the plant and its soil environment. At a closer look, the concept of interface is crucial for the whole system of AM interaction. In their extraradical phase, AM fungi enlarge the nutrient absorptive surface zone around the root, increasing the plant/soil nutrients interface (Leake et al. 2004, Harrison et al. 2002, Smith 2002, Ortas et al. 2004). During their intraradical growth, AM fungi develop an extended contact area with the root cell, which changes structurally depending on the intercellular or intracellular location of the fungus.

Therefore, two interfaces or exchange surfaces are formed: an outer interface, between extraradical

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This work is dedicated to Professor Silvano Scannerini on the occasion of his retirement.

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hyphae and soil, and an inner interface, between intraradical fungal structures and the host plant cells. These two biological surfaces have profound morphological and functional differences. On the one hand the role of external hyphae is to explore the neighboring soil through maximal extension; at the same time the outer interface is actively acquiring nutrients and water from the environment used in the metabolism of both the fungus and the plant. This interface can be described as a continuously extending surface (Smith et al. 2003), with unidirectional transport capabilities.

On the other hand, the inner interface – the focus of this review - develops in a limited space, the root tissues, and time, considering the activity cycle of arbuscules (a few days from their development to senescence). Nonetheless, the inner interface surface is maximized through hyphal branching and tapering. In addition, the 'plant side' completes the interface both structurally and functionally, thus creating a specialized niche where fungal extracellular stimuli are strictly controlled. From a functional point of view, this surface exchanges signals and nutrients to and from each partner. The intraradical interface can thus be described as a dynamically-stable surface (Smith et al. 2003) with bidirectional transport capabilities.

Both surfaces share common features that are mostly related to the specialized and enhanced acquisition capabilities related to the support of plant nutrition. An example of this is the P uptake from soil and its delivery to the plant: these events mirror the roles played by the outer and inner interfaces, respectively (Karandashov & Bucher 2005). The fungus/soil interface is similar to the analogous surface developed by a saprotrophic fungus, while in contrast the symbiont fungus/plant interface resembles the interface established between biotrophic pathogens and their hosts.

One of the most striking characteristics of the inner interface is that it consists of a complex apoplastic compartment that lines each intraradical hypha, even when it penetrates into the plant cell lumen (Scannerini & Bonfante, 1983, Peterson & Massicotte 2004). In fact, even though the first classification principle for mycorrhizas divides them into interactions where only extracellular contacts take place, the ectomycorrhizas, and interactions where the fungus reaches the cell lumen, the endomycorrhizas to which AM belong, in no case is there a direct contact between the plant cytosol and the fungus (Figure 1). So called intracellular hyphae, as well as arbuscules, are always surrounded by a thin layer of plant cell wall materials and an envelope of plasma membrane, safeguarding the host cell integrity, similarly to what is observed in other non-mutualistic biotrophic plant interactions (Panstruga 2003) and mediating, actively and/or passively, the molecular exchanges at the cellular level. AM fungal colonization is, therefore, strictly speaking apoplastic, even if a large percent of intraradical hyphae grow through the cell lumen of epidermal and cortical cells.

The construction of such an organized interface, including a specialized membrane (Harrison 1999, Harrison et al. 2002, Ferrol et al. 2002) and a differential composition of the apoplastic matrix around the intracellular fungal structures (Bonfante 2001), is realized through a vigorous mobilization of the plant cell cytoplasm and to a larger extent of the whole cell activity, ranging from specific gene activation (Gianinazzi-Pearson & Brechenmacher

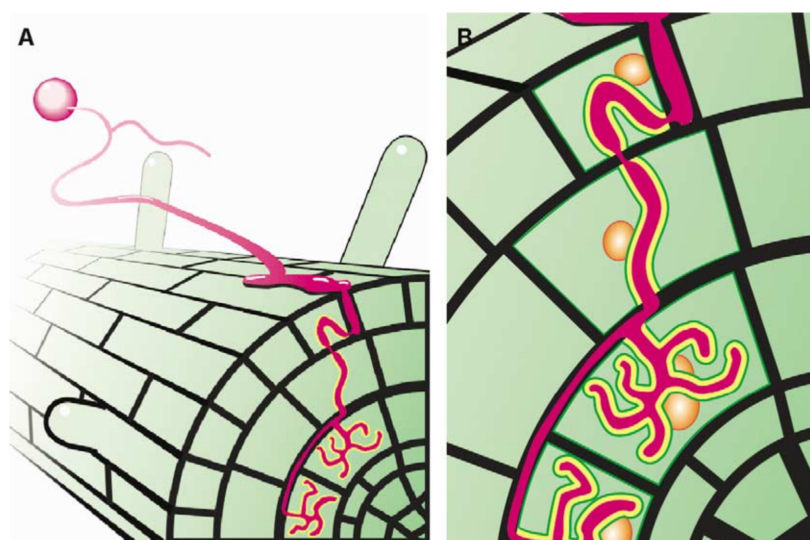

Figure 1. Schematic view of AM colonization in the model legume *Lotus japonicus*. (A) After spore germination, a hypha contacts the root surface, where it differentiates a swollen appressorium and from this a penetration hypha, which then colonizes the epidermis, the cortex and finally develops into branched arbuscules in the cell lumen of the inner cortical cells. (B) Detail of the intraradical fungal growth, where the apoplastic interface is visible (in yellow) lining each intracellular hypha and coated by an invagination of the plant plasma membrane (dark green). Nucleus (orange) repositioning and swelling is present in all the colonised cells, and more evident in the arbuscule containing cells.

2004) to localized cell wall and membrane deposition (Balestrini & Bonfante 2005).

The AM colonization process: An overview

The main aspects of mycorrhizal colonization are briefly summarized here to provide the background for further discussion. Glomeromycota are highly dependent on their hosts and the hyphae germinating from their large asexual spores can only grow for a few days in the absence of the plant. Such presymbiotic hyphae develop in turn, upon the recognition of the host plant, infection units that colonise the root epidermis and cortex (Figure 1).

Presymbiotic hyphae form appressoria on the root epidermis, which in turn form hyphal pegs that cross the epidermal cell lumen, where the interface compartment makes its first appearance. Subsequently, the cortical tissue is colonized with coils and intercellular hyphae which spread the infection, and eventually form intracellular, highly branched structures called arbuscules (Bonfante 1984). Based on the colonization pattern, a classification was proposed by Gallaud at the beginning of the last century and reintroduced by Smith and Smith (1997). The two main categories, named from the plant species where they were first and most typically described, are the *Arum*-type, defined as an extensive intercellular hyphal growth with the development of terminal arbuscules, and the *Paris*-type, mostly showing intracellular coiled hyphae with intercalary arbuscules (Figure 2). In spite of this useful classification, AM interactions show a range of intermediate patterns, depending on the host/fungus combination (Dickson, 2004).

The colonization of the model legume *Lotus japonicus* by *Gigaspora margarita* offers a good example of such an infection with intermediate features. The swollen appressorium, developed at the epidermis surface, initiates an intercellular hypha, which usually separates two adjoining epidermal cells reaching their base. Here, it penetrates the radial wall of the epidermal cell and develops into its lumen (Bonfante et al. 2000). This series of events is a crucial step in the interaction, where reciprocal recognition, localized differentiation of the appressoria, cell wall breaching and intracellular accommodation of the symbiont in a novel apoplastic compartment represent the result of complementary, coordinated strategies performed by both partners to grant the beneficial fungus an access to the root tissues without any damage to the plant.

Once the colonizing hyphal tip has reached the inner wall of the epidermal cell, it grows into the cortical layers, where it originates inter- and intracellular hyphae, with coils and branches. Also in the cortex, the intracellular segments are coated by the interface and a perifungal plasma membrane as described for the epidermis. Finally the hyphae reach the lumen of the inner cortical cells, where arbuscules are formed. The host cell reorganization here reaches its peak. On the one hand the extensive occupation of the cell space by the mass of the arbuscule branches, coupled with the large surface that such a structure develops (Dickson et al. 2003, Toth & Miller 1984), directly influences the complexity of the interface compartment and of the plasma membrane that borders it. On the other hand, arbuscules are the main site of nutrient exchanges, and the molecular mechanisms that mediate them are set up by the time the arbuscule achieves its full development (Rausch et al. 2001, Hahn & Mendgen 2001).

Arbuscules are ephemeral structures: their tips rapidly collapse and in some Glomaceae septa are produced to separate the vital compartments from the apical senescent ones (Bonfante 1984). As discussed previously, the mode of colonization depends on the host and on the fungus: unlike *Gigaspora*, for example, *Glomus* hyphae show fusion events with hyphae of the same isolate during their presymbiotic phases (Giovannetti et al. 2003). Once inside the root, *Glomus* produces long intercellular ‘runner’ hyphae which not only distribute the infection but also anastomose, creating bridges between the root layers and probably reinforcing the vigour of the infection unit.

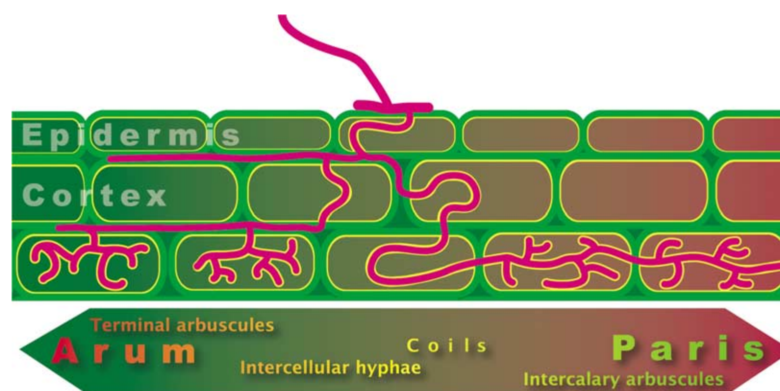

Figure 2. Diagram of the *Arum-Paris* continuum in different AM associations. Different intraradical fungal structures characterize each extreme of the gradient, while shared features are indicated in the central area.

Epidermal and cortical cells: Sharing the job

Only two cell types, epidermal and cortical, can be ‘mycorhizal cells’. Meristems and differentiating tissues are never colonized as well as the endodermis, the vascular tissues, and specialized cortical cells, such as idioblasts or those containing raphides or phenols. The mycorrhization process can be divided into two main steps: epidermis colonization and cortex colonization. During epidermis colonization – ranging from molecular dialogue between the partners to actual fungal penetration across the epidermal cell – the final direction of fungal development is vertical, towards the center of the root. By contrast, upon cortex colonization, a completely different developmental program is set up by the fungus, on one hand aimed to spread the infection horizontally across the root and on the other culminating with the establishment of a functional symbiosis through the construction of arbuscules. Such a discrimination also highlights the cell-specific response of the plant. Root epidermis is the first barrier met by all soil microorganisms during their colonization attempts and it is therefore likely the site of recognition mechanisms as well as welcoming/defense responses (Parniske 2004, Bonfante et al. 2000). By contrast, once the colonization has overcome this first checkpoint and reached the inner tissues, a different set of responses is organized by the plant, allowing extensive intra- and extracellular fungal growth and dramatically reorganizing the cytoplasm (Genre & Bonfante 1998), plastid distribution (Hans et al. 2004), and phosphate transport (Karandashov & Bucher 2005) of the arbuscule-containing cell.

Most research has focused on the second level of the interaction, leaving almost unexplored, until recent years, the epidermis colonization, in spite of its supposedly crucial role for the set up of the interaction. One possible justification for this differential attention is the fact that arbuscules are the main site of nutrient exchange, and therefore the most active and attractive step of the interaction. Nevertheless studying arbuscules is complicated by their deep intraradical location as well as by the variable time lapse between root inoculation and actual symbiosis establishment. This shifted most research towards mature mycorrhizal roots, where the whole range of fungal structures could be observed with ease and with a certain degree of repeatability. As a consequence, epidermal colonization was seen as a step associated with simple interface compartments – with a different composition compared to the periarbuscular ones (Balestrini et al. 1996) – but without any other recognizable cell reaction.

On the contrary, recent technical advances, first of all the production and screening of mycorrhizal mutant plants, gave proper weight to the role of epidermal cells in the early establishment of the AM interaction. Most of the novelties came from the study of legumes, where the wider knowledge of *Rhizobium* interaction led the way (see Parniske 2004 for a review). This approach showed that a number of mutations affected both symbioses, thus demonstrating that they shared at least in part a common genetic pathway (Kistner & Parniske 2002).

In vitro mycorrhization of root organ cultures (Chabot et al. 1992, Chabaud et al. 2002) was another crucial advance that allowed the direct observation of the interaction *in vivo* (Figure 3), avoiding the earlier destructive methods. Being able to follow the whole process of root colonization also led to the study of plant responses before fungal contact, highlighting the presence of a molecular dialogue between the partners, so far completely unexplored (Kosuta et al. 2003).

Altogether these observations raised the role of epidermal cells from a passive barrier to an active checkpoint where signal exchanges and a strong control of the colonization were performed (Novero et al. 2002). This does not mean that the knowledge gap between cortical and epidermal cell responses has narrowed, but a strong incentive has been given to orient research in this direction. In fact epidermal cells are also much more accessible in living roots. It is therefore expected that more information will be drawn from the study of the epidermal step of AM colonization.

An example could be the interface construction. Morphological data report two extreme steps: (i) extracellular hyphae (or appressoria) versus (ii) interface-coated intracellular hyphae that traverse the cell lumen – and analogue steps are reported for arbuscule formation. Based on these observations, most authors hypothesize that the host plasma membrane invaginates and proliferates around the

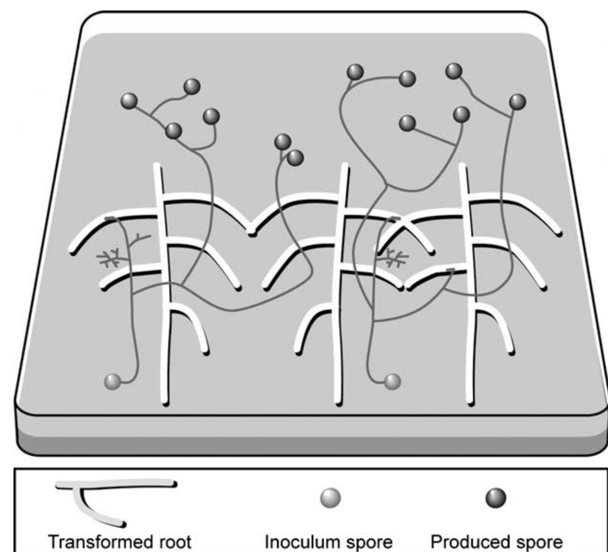

Figure 3. Scheme of *in vitro* root organ culture colonized by an arbuscular mycorrhizal fungus. Negative gravitropism of *Gigaspora* germ tube is exploited to obtain a spore production compartment in the upper part of the Petri dish.

developing fungus (e.g. Bonfante 1984, Ferrol et al. 2002). Recent data, though, obtained by using vital tags for plant cytoskeleton, endoplasmic reticulum and plasma membrane, coupled with the mentioned *in vitro* culture of mycorrhizal transformed roots, report unexpected features. Prior to fungal penetration, epidermal cells markedly reorganize and build an apoplastic track across the cell lumen, through which the colonizing hypha will grow (Genre et al. Submitted). Although these observations need to be repeated with more AM plant and fungal species, they suggest that the plant side of the interface is built in advance, showing how *in vivo* investigations can reveal fundamental and unexpected events in the interaction, and raising new questions, e.g., concerning the accommodation of arbuscules or the analogy with *Rhizobium* infection threads.

Mutant plants as a tool to understand the genetics of plant/fungus compatibility

Plant mutants unable to form AM are a powerful tool to identify genetically defined steps in the development of the symbiotic interaction. The genetic dissection of AM development has been pioneered by the isolation of pea mutants impaired in AM symbiosis. These mutants were initially identified through their altered root nodule symbiosis with *Rhizobium*. Subsequently, it was found that a subset of the nodulation mutants was also affected in the AM symbiosis (Duc et al. 1989). This finding demonstrated an overlap in the genetic programmes for the two symbiotic interactions (Duc et al. 1989, Hirsch & Kapulnik 1998, Peterson & Guinel 2000). Since the isolation of the affected genes from pea was hampered by its large genome size, greater attention has been given to *Lotus japonicus* and *Medicago truncatula* which represent more amenable model legumes to isolate symbiotic mutants. Such genes are collectively referred to as the common *SYM* genes (Kistner & Parniske 2002), and are stimulating also evolutionary studies: the hypothesis is that the relatively recent nitrogen fixing nodules have recruited functions from the more ancient AM symbiosis (Parniske 2004). Molecular analyses of these mutants have recently allowed the identification of three essential components of a plant signaling network: genes encoding for a receptor like kinase (SYMRK), a predicted ion channel (DMI1, in *M. truncatula*) and a calmodulin-dependent protein kinase (DMI3, in *M. truncatula*) have been identified and characterized (Parniske 2004 for a review). A major issue is the positioning of SYM protein activity in the framework of the signaling cascade. A physiological benchmark is the calcium-spiking response, occurring in root hairs 10–30 min after Nod Factor treatment and causing rhythmical oscillations of cytosolic calcium concentration (Oldroyd et al. 2005). This marker allows the positioning of SYMRK (SYM2 in *Lotus japonicus*, DMI2 in *M. truncatula)* as well as DMI1 upstream to the calcium spiking, while DMI3 acts downstream (Figure 4). Thanks to these findings, many of the molecular players that control the 'electrochemical prelude' to the symbiosis (ion fluxes, extracellular alkalinisation) have been demonstrated to be active both in AMs and nodules, even if the experimental evidence of calcium spiking is limited to nodulation.

In the absence of calcium spiking and/or of a corresponding marker in AMs, detailed descriptions of the mutant mycorrhizal phenotypes may provide valuable information. So far only a few *Lotus japonicus* mutants are available. A first set was tested by Wegel et al. (1998) for their interaction with *Glomus intraradices*, and a number of nodulation mutants that were also impaired in the AM symbiosis were identified. Three mutants, *LjSym2, LjSym3* and *LjSym4* all displayed a similar phenotype. Fungal infection was blocked at a very early stage, even though arbuscules were found occasionally. Wegel et al. (1998) concluded that the *LjSym2, LjSym3* and *LjSym4* genes play a role during the early stages of the symbiotic infection, even if they are not required for arbuscule formation.

A new mutant in locus *LjSym4* was further identified (baptised *Ljsym4–2*), where arbuscule formation was never observed in the interaction with two divergent AM fungi, *Glomus* spp. and *Gigaspora* spp. Detailed microscopy of the *Ljsym4–2* phenotype, combined with immunolabelling to determine a few cell wall components, revealed that epidermal cell penetration by *G. margarita* was associated with localized host epidermal cell death (Bonfante et al. 200). However, the strategy of epidermal penetration by *G. margarita* was identical for the *Ljsym4–2* mutant and the parental line, with appressoria, hyphae growing between two epidermal cells, and penetration of epidermal cells through their anticlinal wall. The symbiosis with *Mesorhizobium loti* was also affected, since normal root hair curling and infection threads were not observed, while a *nodC*-dependent deformation of root hair

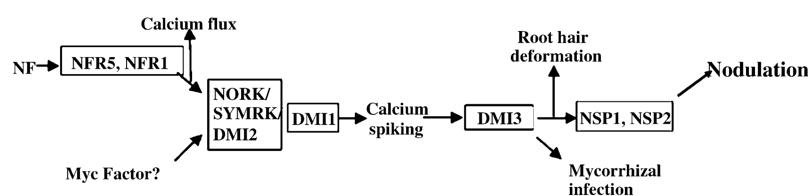

Figure 4. The Nod factor and mycorrhizal signaling pathways. Modified from Oldroyd et al. (2005).

tips indicated that nodulation factors are still perceived by the *Ljsym4–2* mutant. These observations defined a novel genetically-controlled step in AM colonization. Although rhizobia penetrate the tip of root hairs and AM fungi access an entry site near the base of epidermal cells, the *LjSym4* gene is necessary for the appropriate response of this tissue to both microsymbionts. This gene has been recently found to be encoding for a potential ion channel acting upstream intracellular calcium variations (Imaizumi-Anraku et al. 2005) and surprisingly localized in the plant cell plastids. On the basis of these findings and on the mycorrhizal phenotype, we can conclude that *LjSym4* is required for the initiation or coordinated expression of the host plant cell's accommodation program: when missing, the fungus – thanks to its physical pressure – can penetrate the epidermal cell, but breaking down the plant plasma membrane, generates an incompatible interaction (Bonfante et al. 2000). Further genetic analysis suggested that also the characteristic 'opening' resulting from the separation of two adjacent epidermal cells is an active process involving the *Lotus japonicus LjSym15* gene (Demchenko et al. 2004).

In conclusion, mutants are excellent tools to decipher the molecular genetics underlying the colonization process and the establishment of a mycorrhizal cells. However, it is surprising to note that all the data are so far limited to legumes. The next challenge will be to understand whether *Sym* genes are also present in all the mycorrhizal non legume plants. Some tomato mutant plants have already been described as non mycorrhizal (Barker et al. 1998). In some cases they seem to respond differently to AM fungal species: direct screening efforts resulted in the identification of a tomato cv. Micro-Tom mutant M20, which was impaired in its ability to support the pre-mycorrhizal infection (pmi) stages. The Myc- phenotype of the M20 mutant was a single mendelian recessive trait transmitted for nine generations (David-Schwartz et al. 2003). Interestingly some lines were resistant to infection by isolated AM spores of *G. margarita*, while formation of *Glomus intraradices* and *G. mosseae* intraradical structures were normal, as on wild-type (WT) plants (Bonfante and Novero, unpublished results).

On the other hand, the group of Kapulnik demonstrated that soluble factors released from roots of the pre-mycorrhizal infection (pmi) Myc- tomato mutant M161 delayed the proliferation of *G. intraradices in vitro* and inhibited hyphal tip growth of *G. gigantea* and *G. intraradices* (Gadkar et al. 2003). This observation is quite interesting when compared to the recent characterization of the bioactive molecules released by *Lotus japonicus* (Akiyama et al. 2005) as sesquiterpenes (Figure 5): it raises in fact the question whether the mutant legumes release exudates which are affected in their composition and may have some inhibitory effect.

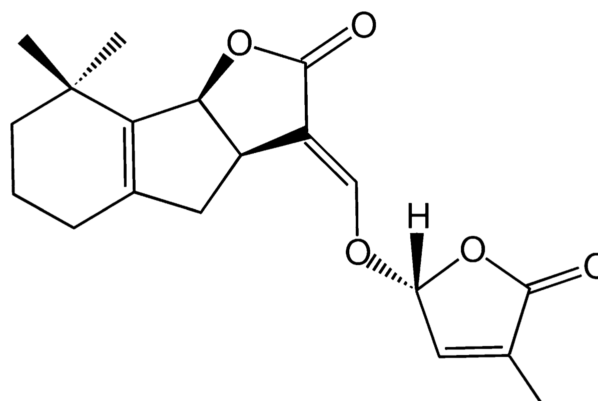

Figure 5. Chemical structure of 5-Deoxy-stirgol, the branching factor isolated from *Lotus japonicus*. Modified from Akiyama et al. (2005).

Plant cell responses to fungal colonization

Biotrophism, literally live-feeding, implies that the host organism provides nutrients to its guest through living tissues. This means that when intracellular colonization takes place, this does not damage host cell integrity. It is not surprising that in all cases of intracellular symbioses, the guest organisms are confined into specialized membrane-bordered spaces, and in this respect AM interaction is not an exception (Parniske 2000).

Nevertheless, hosting single bacterial cells (even several of them) within membrane vesicles may be seen as a brilliant evolutionary modification of endocytosis mechanisms, similarly to what is hypothesized for the acquisition of DNA-containing organelles into eucaryotic cells, and of some secondary endosymbionts (Lucentini 2005). By contrast, the ability to confine an organism as complex as a fungus into a living plant cell, shows to what extent the symbiotic relationship ranges. In fact intracellular AM fungal hyphae can be as thick as 20 μm or branch into the fine arbuscule digitations that occupy the majority of the cell lumen, growing at relatively high rates, keeping their connection through the root tissues and with the extraradical mycelium, potentially developing vigorous osmotic pressure: all together a rather hard guest to deal with, although beneficial.

The impact of mycorrhizal fungi on the root cells (establishment of an intracellular interface, fragmentation of the central vacuole and movement of the nucleus and other organelles towards the fungal branches) has promoted investigation on the role of cytoskeleton as a key structure that allows root cells to be colonized by AM fungi (Timonen & Peterson 2002, Takemoto & Hardham 2004). Cytoskeletal elements provide in fact a network of tracks and highways along which molecules and organelles move around the cell. This function is of particular importance during biotrophic plant/fungus interactions, which require reorganization of the infected cell as well as the development of a contact area for

uni- or bidirectional signaling and nutrient exchanges (Bonfante 2001). Tobacco plants transformed with the promoter of an alpha-tubulin gene fused to GUS have shown that, in roots, the gene is only expressed in the meristem and in colonized cells (Bonfante et al. 1996). This new transcriptional activity is mirrored by a different cytoskeletal organization. An increase in complexity of the microtubule arrays is observed in fact in infected cells (Genre & Bonfante 1997, Blancaflor et al. 2001), where microtubules run along large intracellular hyphae, or connect hyphae to each other and to the nucleus. Substantial changes are also found in the actin microfilaments: they closely follow the fungal branches and envelope the whole arbuscule in a dense coating network, supporting the idea that actin cytoskeleton is closely linked to the perifungal membrane (Genre & Bonfante 1998). Interestingly, these data agree with the observations of Uetake et al. (1997), who were the first to investigate cytoskeletal rearrangement in orchid cells following fungal colonization. In addition, gamma-tubulin labelling revealed microtubule organizing centers (MTOC) along the nuclear envelope and along the host membrane that surrounds the plant/fungus interface (Genre & Bonfante 1999).

When the cytoskeleton of *Ljsym4–2 Lotus* mutant was investigated, a strong disorganization in microtubules and actin microfilaments was observed in the cells where the fungus penetrates in the absence of the proper accommodation process (Genre & Bonfante 2002). These data provide an important confirmation to the hypothesis that the cytoskeleton with its different molecular components is one of the key factors in the developmental programs which allow compatible interactions.

Cytoskeleton can thus be convincingly postulated as a part of the interface, and as a scaffold bridging the cytoplasm with the membrane/wall complex in both partners, mediating a two-way exchange of information between the cellular exterior and the nucleus (Bonfante 2001), and repositioning organelles in the colonized cells. In fact amyloplasts change their distribution in the presence of arbuscules (Hans et al. 2004), closely surrounding the fungal branches, while nucleus moves to a central position and assumes a swollen, lobated shape (Balestrini et al. 1992).

Cell biology approaches based on chemical fixation of samples offer little information on the involvement of other organelles (such as Golgi bodies or endoplasmic reticulum, both responsible for the secretory pathways) in the process of interface construction. On the contrary, recent GFP tagging experiments revealed a direct relationship between endoplasmic reticulum mobilization and interface compartment assembly (Figure 6, Genre et al., Submitted). In addition, expansins have been differentially located in AM roots, being present both in peripheral cell walls of the host and in the interfacial material (Balestrini et al. 2005), suggesting that this class of proteins involved in the cell wall smoothing can be crucial in the accommodation process of the fungus inside the cortical cells. This finding opens new questions on the potential role of expansins in epidermal crossing: both radial and tangential cell walls in fact need to be softened in order to allow fungal penetration.

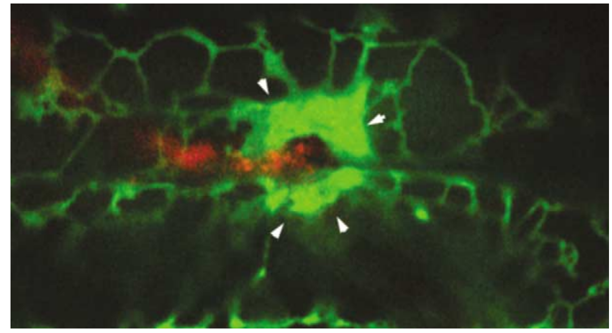

Figure 6. Confocal image of GFP-labelled endoplasmic reticulum (green) in a root epidermal cell of *M. truncatula* showing the assembly of large patches (arrows) underneath the AM fungus appressorium (red). Image width = 110 μm.

By contrast, the development of refined molecular tools has solved a few questions concerning the activation of genes involved in the genesis of the interfacial material (Balestrini & Bonfante 2005). Deposition of cell wall material requires the combined activities of both polysaccharide-synthase and lytic enzymes. Two xyloglucan endo-transglycosilases (XETs) genes have been isolated from *M. truncatula* (Maldonado-Mendoza & Harrison 1998), one being only expressed in mycorrhizal roots. The authors suggest that the gene product may be involved either in facilitating hyphal penetration by allowing localized cell wall loosening or in modifying the structure of xyloglucans in the interface compartment. Lytic and transglycosilation events during cell wall deposition may be facilitated by the interface pH that is acidificated by H^+-ATPase activity (Smith & Smith 1990). The transcript profile of *M. truncatula* roots has been the object of deep investigations during the AM symbiosis with *G. versiforme* (Liu et al. 2003, Journet et al. 2001). By using a cDNA array approach, one gene (MtCel1) induced specifically during the symbiosis was predicted to be involved in cell wall modifications. In mycorrhizal roots, MtCel1 expression is associated specifically with cells that contain arbuscules and, considering the membrane domain, MtCel1 was suggested to be located in the periarbuscular membrane and involved in the assembly of the cellulose/hemicellulose matrix at the interface (Liu et al. 2003).

Taken as a whole, the host cell reorganization can be explained with the need of preserving cell integrity on one hand, and optimizing the exchange activities on the other hand. In particular, reposi-

tioning organelles places them as close as possible to the site where their activity is required: amyloplasts for carbon supply, nucleus for rapid in situ targeting of transcripts, as well as for the displacement of its court of endoplasmic reticulum and Golgi bodies (Neumann et al. 2003), normally mediating localized membrane proliferation and cell wall deposition (Cheung et al. 2003) and therefore possibly involved in the construction of the interface compartment.

The interface as a working place

The process of interface construction is still rather obscure, but the evidence of dramatic cell reorganization after fungal penetration and arbuscule development (Genre & Bonfante 1998, 2002, Blancaflor et al. 2001), together with the complex composition of the interface compartment (Balestrini & Bonfante 2005) strongly suggest that the interface is an active cell compartment not only after its completion, when it mediates nutritional exchanges, but also, probably, during its assembly. This is suggested by the results of Blancaflor et al. (2001), reporting microtubule reorganization in non colonized cortical cells adjacent to arbuscules, explained with the presence of mobile signals operating in the root cortex (Harrison 2005). In addition, the aforementioned data from our group provide direct evidence of epidermal cell reorganization prior to fungal penetration and most likely aimed to interface compartment construction (Figure 6; Genre et al., Submitted). Within the life cycle of a root cell, interface and perifungal membrane are in fact an absolute innovation, a very specific differentiation changing cell morphology, function and metabolism from the status of a storage element accumulating starch to a front-line actor directly interacting with an alien organism through signal and nutrient exchanges. Such a dramatic reprogramming can only find parallels in wound-induced cell division, where de-differentiation of mature cells switches back on their meristematic potentialities. It is interesting to note that mature cell division involves first of all vacuole segmentation, through the organization of the phragmosome, and also in this case organelle repositioning has a fundamental role (Kutsuna & Hasezawa 2002, Panteris et al. 2004).

Once the interface has reached its full functionality, the activity around the fungus/plant contact surface is also extremely intense. Bidirectional transport of phosphate and nitrogen versus carbohydrates through the intraradical interface has been first demonstrated through isotope tracing (Bago et al. 2002, Jakobsen et al. 1992) and then explained by the identification of the responsible enzymes and genes in both the plant (Rausch et al. 2001, Harrison et al. 2002, Hildebrandt et al. 2002, Karandashov et al. 2004) and the fungal cell (Govindarajulu et al. 2005). In addition specific ion pump ATPases have been identified on fungal (Requena et al. 2003) and plant membranes (Ferrol et al. 2002), thus suggesting that fluxes of organic and inorganic compounds are actively controlled on both sides of the interface.

Host plants need to preserve their cell integrity upon fungal colonization. This is a feature that is common to the biotrophic phases of a few pathogenic infections. The activation of typical plant defense mechanisms has been demonstrated with different approaches, ranging from phytoalexin synthesis (Lambais 2000), oxidative burst (Salzer et al. 1999), necrosis (Douds et al. 1998) or salicylic acid accumulation (Blilou et al. 2000, Medina et al. 2003) Fungal countermeasures have been reported as well, such as superoxide dismutase gene activation (Lanfranco et al. 2005). However, notwithstanding the large amount of available experimental data, many basic questions are still open. As Harrison (2005) points out, AM fungi possess surface and cell wall molecules which are common to those from plant pathogenic fungi, but we do not know how AM endophytes avoid triggering plant defence responses. Since the first observations of Spanu et al. (1989), many studies have consistently reported that there is an induction of defence responses during the initial stages of AM development and that these responses decline when the symbiosis develops. A global analysis of gene expression in *M. truncatula* convincingly demonstrates that defence plant genes are down regulated in the mature phases of the plant-fungal interaction (Liu et al. 2003), suggesting that there is suppression mediated perhaps via the arbuscules (Harrison 2005). A fascinating hypothesis is that – during the arbuscule phase – AM fungi may secrete avirulence proteins which can trigger the suppression of plant defense response. Thinking of the dynamics of the fungal development along a mycorrhizal root, we suggest that the potential defence suppression is also related to the interface construction and to the perifungal membrane assembly. Following this line, the interface could be the tool thanks to which the plant-fungal compatibility is reached. The events specific to the arbuscule-containing cells would be more complicated, since at that stage the interface might mediate the senescence and dismantling of arbuscules, another process whose regulation is still far from being understood.

Conclusions and perspectives

Recent AM literature indicates that the role of the mycorrhizal fungus is closely related to its topography: it grows towards the root, responds to active root molecules (Akiyama et al. 2005) by hyphal branching, activates signalling programs when it is still in the rhizosphere, takes up nutrients and delivers them to the plant, playing therefore a very

active role as a biological interface between plant and soil (Leake et al. 2004). By contrast, the role played by the fungus in the development of the fungus/plant interface is much more difficult to define. While it is well acknowledged that the plant *Sym* genes control the accommodation process (Parniske 2004), the fungal genes that could be involved in such a process are completely unknown. Similarly, the cellular morphogenetic mechanisms that are activated during the intraradical and intracellular growth and lead for example to hyphal exploration of the intercellular spaces or to arbuscule differentiation are at least as obscure.

It is largely accepted that AM fungi are asexual, haploid, multinucleated organisms, with a wide range of genome sizes (Hosny et al. 1998). In contrast, a debate is currently focused on the question whether AM fungi have a multigenomic structure or by contrast they have a homokaryotic organization including intra-individual genetic variation (Bever & Wang 2005). Irrespectively of that, the sequencing project for the small sized genome (13,000 Mb) of *G. intraradices*, which is currently under way by the US Department of Energy's Community Sequencing Program (www.Jgi.doe.gov) will provide essential information on the genes that control fungal development.

The conserved morphology of the fungal structures is quite remarkable, when the huge diversity of their host plants is considered. For that reason, it will be very exciting to study whether fungal morphogenesis is under the control of highly conserved genes as are the homeobox genes. Exciting, even if not conclusive, results from Natalia Requena suggest that some genes controlling fungal morphogenesis in AM fungi are closely related to homeotic box in *Drosophila* (Requena 2005).

In conclusion, the process of compatibility between plant and fungus requires a complex cascade of events, some of which are starting to be deciphered. Its last step is the construction of the interface compartment: the high degree of conservation of such a cellular response strongly suggests that this is the result of balanced gene programs activated by both partners and mostly relying on highly conserved genes.

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