

Signal and nutrient exchange at biotrophic plant–fungus interfaces

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Biotrophic interfaces are formed in mutualistic and parasitic plant–fungus interactions. They result from coordinated developmental programs in both partners and represent specialized platforms for the exchange of information and nutritional metabolites. New data on the establishment and the components of functional interfaces have been obtained in a number of ways. First, by isolation of symbiotically defective mutants; second, by characterization of new genes and their products; and, third, by the identification and localization of components of biotrophic interfaces, such as cell-wall proteins, H⁺-ATPases and nutrient transporters.

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Abbreviations

AAT1	AMINO-ACID TRANSPORTER1
AM	arbuscular mycorrhiza
EM	ectomycorrhiza
HXT1	HEXOSE TRANSPORTER1

Introduction

The intriguing structural and functional complexity of biotrophic plant–fungus interactions, as well as their eminent roles both in natural ecosystems and in agriculture, has fascinated biologists for more than a century. Invasion of plant tissue by fungal mycelium and the establishment of a stable relationship result from coordinated developmental programs in both partners that cannot be reproduced in the absence of the other. Mycorrhizal symbioses provide the majority of plants with essential mineral nutrients, whereas the haustoria-forming rust fungi and the powdery- or downy-mildew fungi can cause devastating diseases on all major crop plants. Despite their contrasting impacts on plant health, many of the structural and functional features of these interactions are similar and worth discussing together. In this review, events and components that are associated with the establishment and maintenance of biotrophic interfaces and their functional properties are discussed.

Challenges to the study of biotrophic interactions

A major experimental obstacle to the study of biotrophic interactions is the difficulty of culturing the fungal partners in the absence of their host plants, which often precludes mutant screenings and transformation. The first report of the stable transformation of an obligate biotrophic fungus, barley powdery mildew, has been published only

recently [1*]. Therefore, cultivatable biotrophic fungi (e.g. *Cladosporium fulvum* and *Ustilago maydis*) or hemibiotrophs (e.g. *Colletotrichum* spp.), which have initial biotrophic growth phases before switching to killing host cells, remain indispensable model systems (see below). Increasing attention is becoming focused on model systems involving *Arabidopsis* (infected by powdery and downy mildews) and the legumes *Medicago truncatula* and *Lotus japonicus* (infected by arbuscular-mycorrhizal, mildew and rust fungi). *Arabidopsis* mutants have been isolated that are resistant to powdery mildew infection; their mutations map to four loci that seem to be involved in the establishment of the parasitic interaction [2*]. Extensive mutant screens with the legumes have revealed several loci that control various stages of arbuscular mycorrhiza (AM) establishment; all of the published loci also control the nitrogen-fixing root-nodule symbiosis [3,4].

The search for genes that are induced during biotrophic interactions has been effective in identifying relevant molecular components, which can subsequently be localized by immunocytology. In the *Pisolithus tinctorius*–*Eucalyptus* ectomycorrhiza (EM) interaction, highly abundant fungal cell-wall proteins (i.e. hydrophobins and symbiosis-regulated acidic polypeptides) were identified. These proteins are likely to be involved in cell–cell adhesion at the symbiotic interface [5–7]. Similarly, putative cell-wall proteins at the interface in the AM symbiosis between *Glomus versiforme* and *M. truncatula* have been characterized [8]. In rust fungi, characterization of *in-planta*-induced genes revealed a special role for rust haustoria in metabolite uptake (see below) and in the synthesis of vitamin B1 [9*]. Using an approach that is based on two-dimensional gel electrophoresis and protein microsequencing, at least 16 plasma-membrane proteins were detected that are differently expressed in AM mycorrhizal and non-infected tomato roots. One of the downregulated proteins was identified as a catalytical subunit of the tonoplast H⁺-ATPase [10].

Large-scale gene discovery approaches based on expressed sequence tag (EST) sequencing, hybridization of arrayed DNAs, and differential cDNA-PCR-based techniques are increasingly applied to plant–microbe interactions, including those involving the downy-mildew fungus *Peronospora parasitica* [11,12]. In the *P. tinctorius*–*Eucalyptus* interaction, 17% of 850 mycorrhiza-derived cDNAs revealed significant changes in gene expression when compared to the expression levels in each of the free-living organisms [13].

Signal exchange leading to biotrophic interfaces

For several plant parasitic fungi, the nature of the chemical and physical (e.g. topographical) signals from plants that induce early hyphal differentiation (e.g. appressorium formation) and pathogenesis are fairly well understood, as

are many components of their signal transduction pathways [14–16]. Signals leading to the differentiation of the hyphae of mycorrhizal fungi, however, are not well characterized [17–18]. A branching factor that supports the pre-symbiotic hyphal growth of AM fungi has been partially purified from the root exudates of various AM-forming plants [19]. On the other side, possibly the first-identified fungal molecule that induces symbiosis-related differentiation is the tryptophane betaine hypaphorine. This molecule acts as an auxin antagonist and seems to be involved in the suppression of root-hair elongation during EM development [20*,21*]. However, the production of hypaphorine seems to be restricted to *Pisolithus tinctorius*, and it remains to be seen whether similar molecules play comparable roles in EM interactions. Additional evidence for the role of phytohormones in EM comes from the phenotype of an auxin-overproducing mutant of *Hebeloma cylindrosporum*. This mutant was shown to develop the Hartig net in roots of *Pinus pinaster* more rapidly than the wild-type strain [22].

Evidence to show that the signal transduction pathways leading to root nodule and AM formation overlap is provided by many plant mutants that are defective in both symbioses, and by the common induction of nodulin genes such as ENOD40 [23]. On the other hand, divergent regulatory mechanisms in response to these two symbioses were observed in a study on chitinase gene expression in *M. truncatula*. Similar classes of chitinases were induced in response to nodulation and infection by pathogenic fungi, but not by AM colonization; the AM interaction led to the induction of a mycorrhiza-specific class of chitinases [24**].

Signal exchange during the advanced stages of a biotrophic interaction is very difficult to study and requires the use of appropriate mutants or transformed lines. In *Ustilago maydis*, promoter studies have identified cis-acting sequences that are controlled by putative plant factors. An upstream activating sequence that is essential for the glucose-dependent expression of PHEROMONE RESPONSE FACTOR1 (PRF1), a central regulator of pathogenesis, was identified and the corresponding binding protein purified [25]. Similarly, a promoter region was delimited from a gene induced during the interaction with maize, *mig1* (*maize-induced gene1*), that was required for high expression during *in planta* growth [26*].

Maintenance of the biotrophic lifestyle

Plants are able to respond to pathogen attack quickly through the induction of various defense mechanisms, including programmed cell death. In contrast, only weak or transient defense responses are observed after infection with biotrophic fungi. Such weak defenses benefit both partners in mutualistic symbioses but are to the detriment of the host in parasitic interactions. How is the voracity of the defense mechanisms regulated, and how are stable biotrophic relationships established? A number of mechanisms have been proposed to be involved.

First, the masking or degradation of elicitors. In *Colletotrichum* spp. and rust fungi, the elicitor-active fungal wall polymer chitin is present at the surface of germ tubes and appressoria, but not in hyphae that invade the host tissue. A differentiation-induced chitin deacetylase has been proposed to be responsible for this phenomenon [27], but this needs to be confirmed by mutant analysis. For EM, experimental evidence suggests that the degradation of fungal chitin elicitors is carried out by plant chitinases [28,29].

Second, nutrient deficiency of infected cells. In the absence of sufficient carbon assimilation, genes for quantitative resistance fail to be expressed [30]. Also, powdery-mildew-infected cells are rendered accessible to infection by another, incompatible pathogen under low sugar conditions [31].

Third, the suppression of host defenses. No physiological suppressors from biotrophic interactions with fungi are known yet, although oligogalacturonide oligomers have been chemically and enzymatically released from wheat leaves that suppress elicitor-induced defense reactions [32]. Glycopeptides (called suppressins) from the necrotrophic pea pathogen *Mycosphaerella pinodes* [33] and cyclic β -glucans produced by the bacterial symbiont of soybean *Bradyrhizobium japonicum* [34] are examples of suppressors of plant defense responses.

Further evidence for the existence of fungal regulators of the biotrophic life-style comes from the phenotype of knock-out mutants. In *Cladosporium fulvum*, disruption of the gene for the extracellular protein2 (ECP2) leads to reduced pathogenicity and the elicitation of stronger defense reactions in the host plant, tomato [35]. In *Colletotrichum gloeosporioides*, mutations in the CgDN3 gene are nonpathogenic, but give rise to the formation of necrotic lesions on their host plant *Stylosanthes* after penetration, indicating that CgDN3 is required to avert a hypersensitive-like host response [36]. The phenotype of a nonpathogenic *C. lindemuthianum* mutant that is affected in the CLTA1 gene indicated that this mutant is defective in the transition from the biotrophic to the necrotrophic stages of pathogenic development that usually occurs in the wild type [37**]. Interestingly, CLTA1 belongs to a class of zinc-containing transcriptional activators and could, therefore, be a regulator that controls this transition. A mutant of the pathogenic fungus *Colletotrichum magna* was isolated that had lost its ability to cause disease but competently colonized cucurbit seedlings [38]. These important papers show that host colonization and the development of disease symptoms can be separated and are under fungal control.

Transport phenomena at biotrophic interfaces

The biotrophic interfaces discussed here control either bidirectional flow of nutrients in mycorrhizal interactions or, in case of endophytic or parasitic interactions, flow that is mainly unidirectional. A summary of our present knowledge of the major components of metabolite transport in

Figure 1

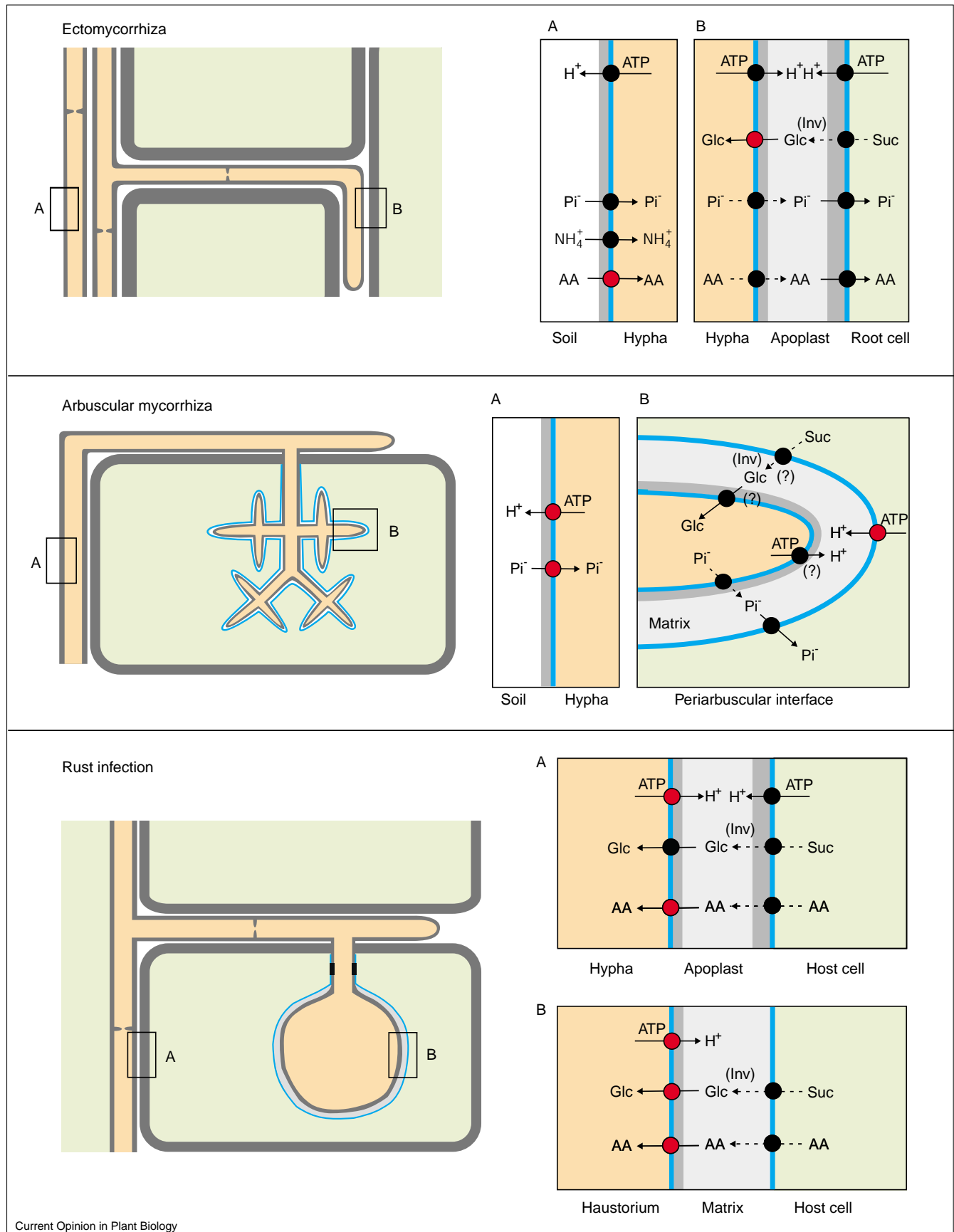


Figure 1 legend

Schematic illustration of postulated transmembrane transport processes occurring at the interfaces of biotrophic plant–fungus interactions. In the arbuscular mycorrhiza, little is known about the membrane proteins involved in nutrient transfer between intercellular hyphae and root cells; this interface is therefore not shown. Membrane proteins (i.e. H⁺-ATPases and transporters) are indicated by filled circles; those that have been cloned and had their localization investigated are filled red. The

uptake carriers shown here are believed to be proton symporters.

Cellular components are distinguished by color: plasma membranes and interface membranes (blue), cell walls (gray), fungal cytoplasm (orange), and plant cytoplasm (green). Dotted lines indicate efflux phenomena for which transporters are postulated but not yet known. AA, amino acids; Glc, glucose; INV, extracellular invertase cleaving sucrose to glucose and fructose; Pi, inorganic phosphate; Suc, sucrose.

the intracellular or intercellular types of interface is illustrated in Figure 1. This summary is based on two assumptions: first, plasma membrane H⁺-ATPase activity indicates proton extrusion across the membrane and the capacity for active metabolite uptake, and second, the symbiosis- and site-specific expression of transporters indicates nutrient uptake by the interface membranes that contain these transporters. Until now, all of the transporters that have been shown or postulated to be involved in symbiosis are proton symporters, which emphasizes the importance of H⁺-ATPases for nutrient uptake.

Mycorrhiza are characterized by the uptake of minerals from the soil by fungal hyphae, the translocation of these minerals within the fungus towards the symbiotic interface, followed by their transfer to root cells. In return, plant carbohydrates are transferred to the fungal symbiont.

In the EM fungus *Amanita muscaria*, a hexose transporter seems to be responsible for symbiotic sugar uptake. This transporter is upregulated both during symbiosis and, when glucose concentrations exceed a threshold of 5 mM, in the free-living state [39]. The regulatory characteristics of a gene encoding an amino-acid permease from the same fungus indicate that it is involved in the uptake of amino acids from the soil [40]. A putative monosaccharide transporter from the host plant *Picea abies* does not, however, seem to play a specific role in symbiosis [39]. AM fungi form intercellular as well as intracellular interfaces with the host plant. Using acidotropic dyes, intensive staining of the periarbuscular matrix indicated that this surface represents a highly acidic compartment [41*]. This acidity probably results from H⁺-ATPase activity in the flanking membranes, which provides the driving force for phosphate uptake by the arbusculated cell. Several PCR fragments from putative *Glomus mosseae* H⁺-ATPase genes have been isolated [42], and activation of one of these genes during symbiosis has been observed in hyphae growing outside of the root (N Requena, personal communication). In tobacco roots, two out of seven tested plant H⁺-ATPase genes were found, using promoter::β-glucuronidase (GUS) fusions, to be induced by AM symbiosis. In addition, immunocytochemical analysis of these roots detected H⁺-ATPase in the periarbuscular membrane but not in uninfected cortical cells [43**]. A high-affinity phosphate transporter from *Glomus* seems to be responsible for the uptake of phosphate by hyphae growing in the soil [44]. The *Lycopersicon esculentum* PHOSPHATE TRANSPORTER1 (LePT1)-encoded

transporter from tomato also appears to be involved in symbiotic phosphate translocation, as indicated by the high levels of LePT1 transcripts that were detected by *in situ* hybridization in arbusculated cells [45]. The site of carbohydrate translocation towards AM fungi is unclear. The absence of measurable ATPase activity in the arbuscular membranes, however, suggests that it might occur mainly via intercellular hyphae [17].

Although most of the parasitic mycelium of rust fungi consists of intercellular hyphae, the ultrastructure and location of haustoria within host cells suggest their special role in nutrition. The haustoria of powdery mildew, which are the only fungal structures to penetrate the host cells, are thought to have a similar role. Consistent with this view is the increased H⁺-ATPase activity in rust haustoria compared to that in spores and germlings, and the expression patterns of genes encoding two amino-acid transporters (AAT1 and AAT2) and a hexose transporter (HXT1) in haustoria of the broad bean rust fungus, *Uromyces fabae*. Although AAT1 is expressed in haustoria and intercellular hyphae, the transcripts and the proteins encoded by AAT2 and HXT1 have been detected only in haustoria [46,47]. Heterologous expression in transgenic yeast and *Xenopus* oocytes demonstrated that HXT1p is a proton co-transporter specific for glucose and fructose [48]. Taken together, these findings support the idea that haustorial interfaces represent the preferred route for the translocation of host metabolites into rust fungi.

Conclusions

The investigation of biotrophic plant–fungus interactions on a molecular level is still at its beginning and remains a formidable task. Rapidly increasing collections of genes obtained by large-scale sequencing projects, and the application of gene-expression profiling and functional genomics, will allow many regulatory and functional phenomena to be studied comprehensively in the near future. For instance, the picture of how nutrients and minerals flow between plants and fungi will be clarified once the major transporters have been cloned and located at the interface membranes. However, new techniques must be developed for the study of the dynamics of biotrophic interactions, such as metabolic pathways and fluxes, *in situ* [49]. Many central questions remain to be answered. What signals control the formation of symbiotic interfaces? How are metabolites and minerals exported across the membranes of donor cells? What properties (e.g. the secretion of

enzymes and toxins, interface components and signaling mechanisms) distinguish biotrophic pathogens from endophytes, from hemibiotrophs and from necrotrophs? How do fungal symbionts induce metabolic sinks at the infection sites to ensure nutrient supply from the host plant [50]? What roles do sugars and sugar metabolizing enzymes (e.g. invertases) play in sink induction and in the symbiotic differentiation of the fungus, for instance in AM [51]? What are the roles of phytohormones, such as cytokinins [52] and auxins [21*,53], during symbiosis? How do the symbiotic partners contribute to the modulation of phytohormone levels or activities?

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