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Plant Cell Responses to Arbuscular Mycorrhizal Fungi: Getting to the Roots of the Symbiosis

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INTRODUCTION

Since their colonization of terrestrial ecosystems, plants have developed numerous strategies to cope with the diverse biotic and abiotic challenges that are a consequence of their sedentary life cycle. One of the most successful strategies is the ability of root systems to establish mutualistic and reciprocally beneficial symbiotic relationships with microorganisms. Mycorrhizas, the intricate associations roots form with specific fungal groups, are by far the most frequent of these and represent the underground absorbing organs of most plants in nature (Gianinazzi-Pearson, 1984). Through their function in the efficient exploitation of soil mineral resources and their bioprotective role against a number of common soilborne pathogens, mycorrhizas are instrumental in the survival and fitness of many plant taxa in diverse ecosystems, including many crop species (reviewed in Allen, 1991; Bethlenfalvay and Linderman, 1992).

Several kinds of mycorrhizal associations can be distinguished according to their morphology and the plant and fungal taxa concerned. They fall almost exclusively into two broad groups: (1) the ectomycorrhizas of woody Angiosperms and Gymnosperms, in which Basidiomycetes, Ascomycetes, or Zygomycetes develop intercellular hyphae from a mycelial sheath covering the surface of short lateral roots; and (2) the endomycorrhizas, characterized by intraradical mycelium growth and intracellular fungal proliferation, which are formed by Basidiomycetes in the Orchidaceae (orchidoid mycorrhiza), Ascomycetes in the Ericales (ericoid mycorrhiza), and Zygomycetes in most other terrestrial plant taxa (arbuscular mycorrhiza; reviewed in Harley and Smith, 1983).

Plant compatibility with mycorrhizal fungi is a generalized and ancient phenomenon. Species in >80% of extant plant families are capable of establishing arbuscular mycorrhiza (AM), and fossil evidence suggests that symbioses of this kind existed >400 million years ago in the tissues of the first land plants (Pirozynski and Dalpé, 1989; Remy et al., 1994). As such, the ability of plants to form AM must be under the control of mechanisms that have been conserved in new plant taxa as they appeared during evolution. This compatibility also implies that selective recognition processes in plants discriminate between beneficial and harmful microorganisms and that the essential genetic determinants for AM establishment are common to an extensive part of the plant kingdom.

In contrast to their extremely wide host range and despite their ancient origins, only six genera of fungi belonging to the order Glomales of the Zygomycetes have evolved the ability to form AM (Morton and Benny, 1990). Interactions between an AM fungus and a plant begin when a hypha from a germinating soilborne spore comes into contact with a host root. This step is followed by induction of an appressorium, from which an infection hypha penetrates deep into the parenchyma cortex (Figure 1A), where inter- and intracellular proliferation of mycelium is intense. Here, fungal development culminates in the differentiation of intracellular haustoria, known as arbuscules (Figure 1B). These fungal structures, which establish a large surface of contact with the plant protoplast, are attributed a key role in reciprocal nutrient exchange between the plant cells and the AM fungal symbionts (Smith and Smith, 1990). However, arbuscules are ephemeral structures, and an individual arbuscule reaches full development within several days, after which it begins to senesce (Alexander et al., 1988). AM development continues within a root system as the fungus spreads to newly emerging roots. In this way, fungal colonization occurs concomitantly in different roots in an unsynchronized manner.

There have been extensive studies of AM over the past two decades, but information concerning the cellular and molecular aspects has remained largely descriptive. This is partly due to the complexity of the biological system and to the status of AM fungi as obligate symbionts; thus, there is a lack of knowledge about their physiology and genetic constitution. However, recently, significant steps have been made toward unraveling plant mechanisms governing AM symbioses through approaches using mycorrhiza-defective plant mutants, molecular cytology and molecular biology techniques, and comparisons of plant interactions with microorganisms in other systems. This review highlights current knowledge about the colonization process in AM, the properties of the ensuing symbiotic interface, the plant genes involved, and the molecular nature of host plant responses to AM fungi. The impact of progress in these different areas is discussed in relation to

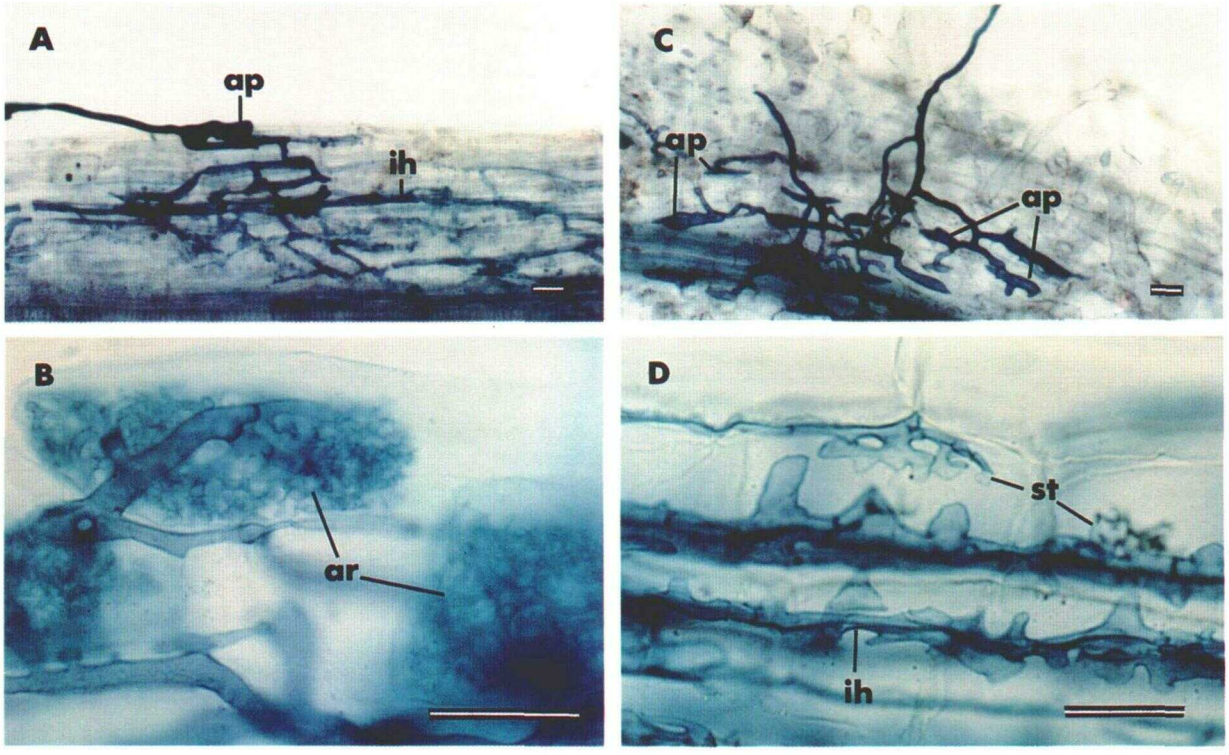

Figure 1. Phenotypes of Interactions between Pea Roots and an Arbuscular Mycorrhizal Fungus (*Glomus mosseae*).

(A) and **(B)** Wild-type genotype (cv Frisson). **(A)** shows an infection hypha penetrating outer root tissues from an appressorium (ap), and intercellular proliferation (ih) in the cortical parenchyma where intracellular, highly branched arbuscules (ar) form **(B)**.
(C) Myc^{-1} mutant (P2) of cv Frisson. Appressoria (ap) differentiate at the root surface but do not develop infection hyphae.
(D) Myc^{-2} mutant (RisNod24) of cv Finale. Intercellular hyphae (ih) colonize the cortical parenchyma of roots but only form stumpy branches (st) inside root cells. Fungal tissue was stained for 15 min at 90°C in 0.1% trypan blue after clearing roots for 1 hr at 90°C in 10% KOH. Bars = 20 μm.

perceiving not only the basis of widespread plant compatibility with AM fungi, but also the relevance of this symbiosis to the understanding of other plant–microbe interactions.

ROOT COLONIZATION PROCESSES

Recognition and Cell Response

The cellular adaptation necessary for morphological integration and reciprocal functional compatibility between the plant and fungal cells in AM must depend on finely tuned recognition processes and on highly evolved molecular coordination between the two. Signal exchange and recognition between host plants and AM fungi initiate before they come into physical contact. Metabolites exuded by host roots specifically enhance spore germination and fungal growth, as compared with root exudates from nonhost plants, which do not exert this effect. The active plant molecules have not yet been identified. Although flavonoids have been proposed as candidates (reviewed in Phillips and Tsai, 1992), they are not absolutely essential to host–fungus interactions (Bécard et al., 1995).

The earliest defined recognition phenomenon in the fungus is host-induced hyphal branching followed by the formation of appressoria at the root surface. These responses can occur as early as 36 hr after inoculation of the host plant (Giovannetti et al., 1994). Cells of the epidermis and hypodermis in contact with these first infection structures show no significant cytological modifications or responses typical of plant defense (Gianinazzi-Pearson et al., 1996a), and the fungus invades the root intercellularly or intracellularly. Plant reaction to a hypha crossing an epidermal or hypodermal cell is apparently restricted to the synthesis of a host-derived membrane and the deposition of cell wall material continually around the fungus. Fungal development is extremely limited in outer root tissues (Figure 1A), and hyphae quickly progress toward the inner cortical parenchyma, where the mycelium proliferates intercellularly along the root. Here, intercellular hyphae branch into root cells, where they differentiate into the highly ramified arbuscules characteristic of this symbiosis (Figure 1B). The restricted pattern of arbuscule development, which is a fea-

ture common to all AM symbioses, implies some sort of plant control over fungal morphogenesis within root tissues, perhaps specific to cortical parenchyma cells. Similar plant control over the development of microbial symbionts is seen in other systems (reviewed in Long, 1996; Pawlowski and Bisseling, 1996, in this issue).

Extensive cytological modifications are induced in arbuscule-containing cells, and these are reminiscent of those seen in undifferentiated meristematic cells or cells entering the cell cycle. The vacuole decreases in size as the relative volume of cytosol increases and cell organelles (mitochondria, plastids, dictyosomes, and endoplasmic reticulum) proliferate. Concomitant with arbuscule development and reactivation of the cytoplasmic compartment, the plant nucleus moves to the center of the cell (Figure 2A) and undergoes hypertrophy (Balestrini et al., 1992). There is evidence that such reorganization of host cell contents requires synthesis of cytoskeletal components because it is accompanied by the transcriptional activation of a plant α-tubulin gene in the colonized cells (Bonfante et al., 1996). The plant cell nucleus is characterized by enhanced fluorochrome accessibility, increased nuclease sensitivity, and chromatin dispersion. All of these modifications reflect an increase in chromatin decondensation, which is a sign of greater transcriptional activity of the plant genome in arbuscule-containing cells (see below; Berta et al., 1990; Sgorbati et al., 1993).

The branching progression of the fungus into the host cell provokes de novo synthesis of the periarbuscular membrane (PAM). The PAM is derived from the peripheral plasma membrane and completely surrounds the arbuscule (Figure 2B). The resulting surface of contact between the plant protoplast and the fungal cell creates the symbiotic interface, a specialized apoplastic zone for nutrient exchange between the mycorrhizal symbionts.

The Symbiotic Interface: From Structure to Function

Extensive biosynthesis of the PAM to accommodate the developing fungus is associated with wall-building activities, and as in epidermal or hypodermal cells, wall material is deposited against the invading fungal hyphae by the adjacent plant protoplast (Dexheimer et al., 1979; Bonfante-Fasolo et al., 1990; Bonfante, 1994; Balestrini et al., 1996; Gianinazzi-Pearson et al., 1996a). However, in cortical parenchyma cells, this wall material progressively thins out around the growing fungus to form a matrix of disorganized fibrils that separates the PAM from the arbuscule wall, creating an interfacial zone of 100 nm or less. The PAM has a neutral phosphatase activity, which is a marker of glycosylation, and some of the interfacial matrix molecules have been identified as typical plant cell wall components (Table 1; Jeanmaire et al., 1985). The variation in interface components between different plants reflects variations in their cell wall makeup. It therefore appears that the symbiotic plant membrane continues to synthesize cell wall precursors, even though structured wall material is not deposited in the interface. The impairment of wall edification may be related at least in part to the acidification of this compartment (see the discussion below) or to the presence of polygalacturonase in the arbuscule interface (Peretto et al., 1995). When the arbuscule begins to senesce, the fibrillar material encapsulates the collapsed fungal structures. Subsequently, the host nucleus, cytoplasm, and vacuole recover their aspect before cell invasion (Jacquelinet-Jeanmougin et al., 1987), demonstrating the remarkable developmental plasticity of cortical parenchyma cells in roots.

The significance of the molecular composition of the symbiotic interface is at present a matter of debate. The plant cell wall components may simply create a barrier that prevents direct physical contact between the hyphal cell wall and plant protoplast surfaces. However, the detection in this cell compartment of the pathogenesis-related (PR) group-1 protein, which is not deposited around hyphae crossing epidermal or hypodermal cells, may reflect a nonspecific defense response

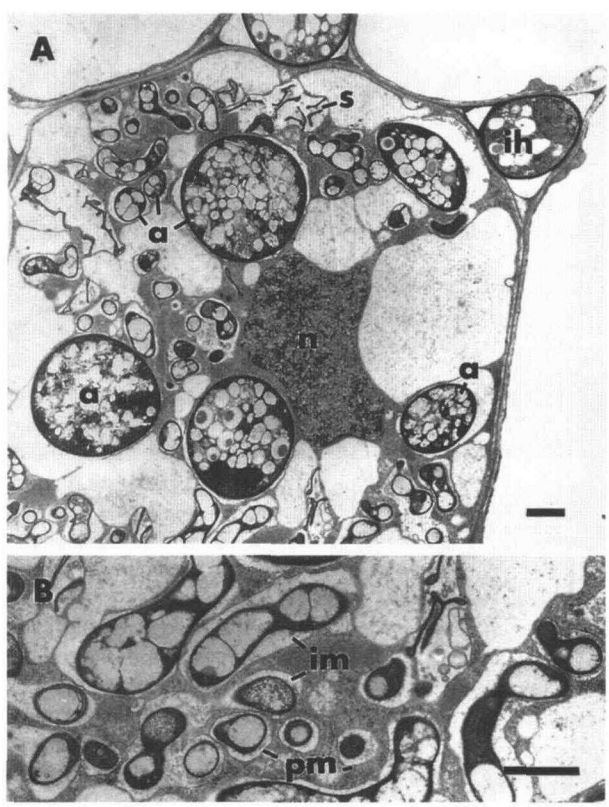

Figure 2. Ultrastructure of Plant–Fungal Relationships in an Arbuscule-Containing Root Cell of *Pisum sativum*.

(A) Cross-section of arbuscule branches (a) surrounded by organelle-rich plant cytoplasm and a centrally positioned lobed nucleus (n). ih, intercellular hypha; s, senescent arbuscule branches.

(B) Detail of fine arbuscule branches surrounded by the periarbuscular membrane (pm) and an interfacial matrix (im).

Bars = 1.5 μm.

Table 1. Plant Cell Wall Components Identified in the Symbiotic Interface

Component	Plant[a]	References
Xyloglucans	+: onion, leek, clover[b] −: maize, tobacco[c]	Dexheimer et al. (1979); Balestrini et al. (1996)
Galacturonic acid	+: onion[b]	Dexheimer et al. (1979)
Nonesterified pectins	+: leek, maize[c] −: pea[c]	Bonfante-Fasolo et al. (1990); Balestrini et al. (1994); Gollotte et al. (1995b)
Cellulose/ hemicelluloses	+: leek, maize[c]	Bonfante-Fasolo et al. (1990); Balestrini et al. (1994)
Hydroxyproline-rich glycoprotein	+: leek, pea, maize[b]	Bonfante-Fasolo et al. (1991); Balestrini et al. (1994)
Glycocalyx oligosaccharide	+: pea[c]	Gianinazzi-Pearson et al. (1990)
Arabinogalactan/ arabinogalactan protein	+: pea[c] +: maize, clover, tobacco[c] −: leek[c]	Gollotte et al. (1995b); Balestrini et al. (1996)

[a] Presence (+) or absence (−) of each component.
[b] Components identified cytochemically.
[c] Components identified immunologically.

that could contribute to the control of fungal spread within the host (reviewed in Gianinazzi-Pearson et al., 1996b). Alternatively, the molecules accumulating in the symbiotic interface may act as signals for recognition between plant and fungus. Furthermore, until the heterotrophic capacities of AM fungi are known, the possibility cannot be excluded that wall precursors also provide a source of reduced carbon to the fungal symbiont (Smith and Smith, 1990).

The chemical form of molecules transferred across the symbiotic interface is not defined. In the context of the whole plant, the mycorrhizal tissue functions predominantly as a fungal sink for carbon supplied as photosynthate and as a source of phosphate transported from soil into the roots via the fungal pathway (reviewed in Harley and Smith, 1983; Jakobsen, 1995; Shachar-Hill et al., 1995). Mechanisms for controlled transport between the mycorrhizal symbionts are largely hypothetical and have been discussed in detail by Smith and Smith (1990) and Smith et al. (1994). Until experimental evidence arises to the contrary, nutrient exchange can still be assumed to involve passive movement of solutes into the interface followed by active uptake by symbiont cells across membranes (Woolhouse, 1974).

The PAM is not structurally or cytochemically different from the peripheral plasma membrane, and evidence that it is an active membrane comes from the observation of H^+-ATPase and nonspecific phosphatase activities at the symbiotic interface of several plants (Gianinazzi-Pearson and Gianinazzi, 1988; Smith and Smith, 1990; Gianinazzi-Pearson et al., 1991a). H^+-ATPase activity is not observed on the peripheral plasma membrane of cortical root cells, whether or not these are colonized by an AM fungus, or around hyphae crossing epidermal or hypodermal cells. The modified ATPase distribution in mycorrhizal roots of leek is mirrored by an overall and stable hyperpolarization of plant membranes, as compared with nonmycorrhizal roots (Fieschi et al., 1992). In nonmycorrhizal roots, immunologically detectable plasma membrane H^+-ATPase is located in the root cap, epidermis, and stele, but it is not present in cells of the cortical parenchyma (Parets-Soler et al., 1990; Stenz et al., 1993; Bouché-Pillon et al., 1994). This distribution has been corroborated by studies of the expression of two H^+-ATPase genes in promoter–β-glucuronidase (GUS) fusion assays: plasma membrane H^+-ATPase promoters are apparently not active in differentiated root cortical parenchyma (DeWitt et al., 1991; Michelet et al., 1994). There is evidence that root plasma membrane H^+-ATPase genes are upregulated during AM interactions. In barley, AM development is accompanied by increased levels of transcripts hybridizing to an H^+-ATPase homologous cDNA clone (Murphy et al., 1996), and promoter–GUS fusion assays in tobacco indicate that at least two H^+-ATPase genes are activated in arbuscule-containing cells (V. Gianinazzi-Pearson, L. Moriau, and M. Boutry, unpublished data).

The major role of the H^+-ATPases at the symbiotic interface should be in driving the two-way nutrient flow occurring across the interfacial apoplast in AM. Loading of phosphate from the interface apoplast into the plant cell will be against a concentration gradient, and the proton motive force created by the H^+-ATPase activity could provide the energy required for the transport of this anion in a symport system (Smith and Smith, 1990). However, increased proton pumping with the activation of H^+-ATPases in the PAM could acidify the interfacial apoplast (Smith and Smith, 1990). The ensuing drop in pH possibly contributes to the wall loosening observed during arbuscule development, either by breaking acid-labile bonds or by activating lytic enzymes in the wall (Navi et al., 1986; Rayle and Cleland, 1992). Finally, modulation of a plasma membrane H^+-ATPase by protein kinases has been invoked in signal transduction that regulates plant defense responses in interactions with fungal pathogens (Xing et al., 1996). The presence of H^+-ATPase activity in the symbiotic interface of AM might then be related to a signal process involved in the weak or partial induction of plant defense responses by AM fungi (see the discussion below).

MOLECULAR GENETICS OF AM

The lack of specificity between the plant and fungal taxa that form AM raises the question of the nature of the genetic deter-

minants involved. Gene-for-gene mechanisms like those postulated to explain specificity in plant–pathogen interactions are unlikely to be active in such extreme examples of reciprocally compatible interactions, where the lack of adverse selection pressures have most likely generated more generalized, multigenic systems. The effects of genotypic variation on AM colonization have been described in a wide number of plant species. A particularly strong genetic basis for colonization ability has been demonstrated in wheat and its ancestral relatives (reviewed in Hetrick et al., 1995; Peterson and Bradbury, 1995). These examples, however, mostly involve polygenic variation. Plant mutants showing alterations in mycorrhiza-forming abilities have been obtained (see below), but technical difficulties in observing large numbers of root systems for defective phenotypes have limited the number of mutants that have been isolated to date. Nonetheless, mutations affecting AM formation have been found in legume species, although only in lines that are also altered in their nodulating abilities.

Isolation and Characterization of Mycorrhiza-Defective Plant Mutants

Almost half of nearly 50 nonnodulating (Nod^-) ethyl methanesulfonate (EMS)–induced isogenic mutants of pea (*P. sativum* cvs Frisson, Finale, and Sparkle) and γ-radiation mutants of *Medicago truncatula* have been found to be completely resistant to AM fungi (Duc et al., 1989; Gianinazzi-Pearson et al., 1991b; Balaji et al., 1994; Sagan et al., 1995). The mycorrhiza-resistant phenotype of these mutants, presently designated Myc^{-1}, is the most frequently found among nodulation mutants. Myc^{-1} mutations specifically affect plant interactions with AM fungi and rhizobia because they are not correlated with changes in the infection patterns of fungal, bacterial, or nematode root pathogens (Gollotte et al., 1993; Gianinazzi-Pearson et al., 1994). Investigations of the genetic system determining the Myc^{-1} character in pea have shown that mutations are each at a single locus and that they belong to three complementation groups (p, c, and a), which correspond to the *sym8*, *sym19* (described by LaRue and Weeden, 1992), and *sym30* nodulation genes (M. Sagan and G. Duc, personal communication). For each complementation group, the mutation is recessive and genetically stable, and the Myc^{-1} trait does not segregate from the Nod^- character in large F_2 progenies. This suggests that one gene controls both characters (Duc et al., 1989; Gianinazzi-Pearson et al., 1991b; G. Duc and A. Trouvelot, unpublished data).

The products of the genes defined by the $Myc^{-1}Nod^-$ mutations must have a fundamental role in establishing mycorrhizal and nodule symbioses because no field populations or laboratory isolates of the microsymbionts have yet been found to infect $Myc^{-1}Nod^-$ pea mutants, whatever the plant growth conditions (Sagan et al., 1993; V. Gianinazzi-Pearson, unpublished data). One exception is the unstable Myc^{-1} phenotype reported in a Nod^- *M. sativa* line (Bradbury et al., 1993), which reverts to mycorrhiza formation under high light conditions. In this case, the Myc^{-1} phenotype may result from alterations in genes involved in normal plant physiological processes, such as sugar synthesis or transport, that are also important for mycorrhiza development.

Initial recognition events occur between Myc^{-1} plant mutants and fungi as appressoria differentiate on the root surface; however, AM development is arrested at this stage (Figure 1C). Because this resistance results from single gene mutations in Myc^{-1} pea mutants, the corresponding wild-type genes could play a role in restricting plant defense responses so that symbiosis can be established (Gollotte et al., 1993; Gollotte, 1994). Myc^{-1} pea mutants challenged with AM fungi deposit abnormally thick cell wall appositions in epidermal and hypodermal cells. These structures contain wall-reinforcing and defense-related molecules, such as phenolics, callose, and PR-1 protein (Gollotte et al., 1993, 1995a; Gollotte, 1994; Gianinazzi-Pearson et al., 1996b), and are reminiscent of the cell wall appositions induced at sites of resistance to pathogen attack (Heath, 1980; Benhamou et al., 1989; Tahiri-Alaoui et al., 1993a; Collinge et al., 1994). It has been postulated that PR-related gene expression in epidermal tissues may contribute to defense in roots (Mylona et al., 1994), but whether resistance to AM fungi in Myc^{-1} mutants is reinforced by activation of other inducible plant defense processes has yet to be investigated.

Two possible functions can be envisaged for the wild-type alleles of Myc^{-1} mutated loci. First, they may be involved in the production of a plant susceptibility factor that is elicited by the fungal symbiont and that negatively regulates the defense response to establish interorganism compatibility, in a manner similar to that postulated for interactions between *Mlo* alleles in barley and the biotrophic fungal pathogen *Erysiphe graminis* (Freialdenhoven et al., 1996). Alternatively, the activated symbiosis-related genes may induce a fungal suppressor of the plant defense system, as has been reported for certain plant pathogens (reviewed in Shiraishi et al., 1991).

Another mycorrhiza mutant phenotype (Myc^{-2}), affected at the stage of arbuscule development, has been identified in two of ~20 EMS-induced low- and late-nodulating mutants ($Nod^{+/-}$) of *P. sativum* cv Finale. Only one recessive and genetically stable mutation is known for Myc^{-2} mutants (the s complementation group; G. Duc and M. Sagan, unpublished data). In these mutants, the stages of appressorium formation, root penetration, and cortex colonization by intercellular hyphae are completed. Although invasion of cortical parenchyma cells was not described in initial investigations (Gianinazzi-Pearson et al., 1991b), more detailed studies have since shown that intracellular fungal growth does occur. However, arbuscule formation is prevented, and hyphal growth is reduced to a few stumpy branches (Figure 1D; Lherminier, 1993; Gollotte, 1994). The cellular processes affected by the Myc^{-2} mutation have not yet been investigated fully, but there is cytological evidence that mycorrhiza function is impaired in Myc^{-2} mutants. This is because ATPase activity is not elicited on the plant membrane at the intracellular host–fungus interface of the

aborted or incomplete arbuscules (Gianinazzi-Pearson et al., 1995). It is therefore possible that the wild-type gene corresponding to the Myc^{-2} mutated locus encodes a component of signaling events leading to reciprocal morphofunctional compatibility between the mycorrhizal symbionts.

The precise roles of the mutated genes in the Myc^{-1} and Myc^{-2} plants have yet to be elucidated. The present indication from the pea system is that different genetic processes are active in the outermost tissues and in the cortical parenchyma of host roots, as defined by the fungal growth phenotypes resulting from the Myc^{-1} and Myc^{-2} mutations, respectively. Wild-type genes corresponding to Myc^{-1} loci may encode proteins that modify defense responses, thus allowing root colonization to proceed, whereas Myc^{-2} loci may be involved in subsequent developmental processes that establish the metabolic specialization of arbuscule-containing cells. Furthermore, the strong link between the genes determining nodulation and mycorrhiza formation in Myc^{-1} pea mutants points to common mechanisms regulating at least part of the plant–microbe interactions in the two symbioses (see the discussion below). There are undoubtedly many more plant genes involved in the establishment of AM. The isolation and genetic analyses of additional plant mutants with a range of mycorrhiza-defective phenotypes will lead to a fuller appreciation of the genetic complexity of systems regulating successful AM symbioses.

Molecular Analysis of AM-Induced Modifications

Plant Defense Responses

Clearly, the plant symbiont exerts a degree of control over AM fungal development, restricting propagation and arbuscule formation to the cortical parenchyma. However, the formation of AM constitutes a massive fungal invasion of plant tissues. It is perhaps not surprising then that the first molecular modifications investigated in relation to the development of this symbiosis were those associated with plant defense responses. The major inducible defense mechanisms that are triggered in plants as a general response to attack by a pathogen are cell wall modifications, enhancement of secondary metabolism, and accumulation of PR proteins (reviewed in Bowles, 1990; Dixon and Harrison, 1990; Collinge et al., 1994; Ryals et al., 1996, in this issue). The spatial and temporal expression of these responses during the formation of AM have recently been described in a detailed review (Gianinazzi-Pearson et al., 1996b). Consequently, only the most salient features are discussed here.

Isoflavonoid phytoalexins are antimicrobial phenylpropanoid derivatives that often accumulate rapidly in plant tissues exhibiting resistance to pathogen attack (reviewed in Hahlbrock and Scheel, 1989; Smith, 1996). Weak, slow, or transient accumulation of phytoalexins, or phytoalexin precursors, has been reported in root systems of AM soybean and *Medicago* species (Morandi et al., 1984; Harrison and Dixon, 1993; Volpin et al., 1995), and the expression of genes encoding the early phenylpropanoid biosynthetic enzymes phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone isomerase (CHI) is enhanced concomitant with isoflavonoid accumulation (Harrison and Dixon, 1993; Volpin et al., 1994, 1995). However, no such changes have been observed in bean, parsley, or potato roots (Figure 3; Lambais and Mehdy, 1993; Franken and Gnädinger, 1994). Furthermore, in mycorrhizal *Medicago* species, there is no increase in transcripts of the late enzyme isoflavone reductase (IFR), which is specific to isoflavonoid biosynthesis, signifying an uncoordinated induction of genes in the phenylpropanoid pathway and contrasting with the coordinated regulation that is a feature of pathogen interactions (Harrison and Dixon, 1993; Volpin et al., 1995). Thus, activation of the phenylpropanoid pathway cannot be considered a general phenomenon linked to AM development.

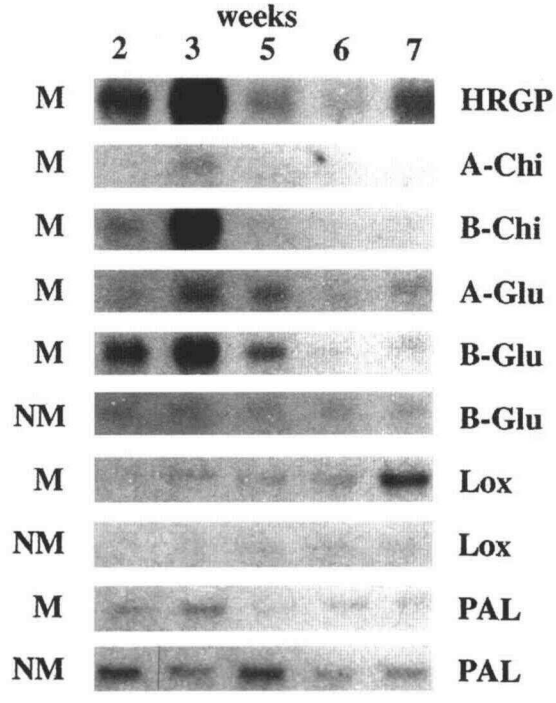

Figure 3. Modulation of Defense Gene Expression in Potato Roots Inoculated with an AM Fungus (*Glomus* sp).

RNA from mycorrhizal (M) and nonmycorrhizal (NM) roots was sequentially probed with ^{32}P-labeled cDNA fragments encoding hydroxyproline-rich glycoprotein (HRGP), acidic (A-Chi) and basic chitinase (B-Chi), acidic (A-Glu) and basic β-1,3-glucanase (B-Glu), lipoxygenase (Lox), and phenylalanine ammonia-lyase (PAL). Total RNA was isolated after 2, 3, 5, 6, and 7 weeks growth of plants, when root colonization in *Glomus*-inoculated plants was 15, 20, 32, 60, and 82%, respectively. An equal amount (20 μg) of total RNA was loaded into each lane. Signals for RNA from NM roots with HRGP, A-Chi, B-Chi, and A-Glu probes were the same as those for B-Glu (A. Tahiri-Alaoui, G. Strittmatter, and V. Gianinazzi-Pearson, unpublished data).

PR proteins involved in the defense response of plants include the acidic or basic chitinases and β-1,3-glucanases, which are antimicrobial hydrolases (Collinge et al., 1994). Enhanced chitinase and β-1,3-glucanase activities coincide with early plant–fungal interactions in AM and strongly diminish as root colonization proceeds (Spanu et al., 1989; Lambais and Mehdy, 1993; Vierheilig et al., 1994; Volpin et al., 1994). This pattern of induction is also apparent at the level of transcript accumulation, suggesting that the enzymes are transcriptionally regulated during symbiosis development (Figure 3; see also Lambais and Mehdy, 1993). Differential activation of genes may reflect partial elicitation of a general plant defense response to early stages of fungal invasion that is subsequently repressed as the symbiosis becomes established. Chitinase isoforms different from those related to plant defense response to root pathogens are activated in the AM of several plants in addition to constitutive root enzymes (Dumas-Gaudot et al., 1992; Dassi et al., 1996). The site of activity and the role of the mycorrhiza-related isozymes in the symbiosis have yet to be determined. However, the mycorrhiza-related chitinase isoform can be weakly active in incompatible interactions in myc^{-1} mutants (Dumas-Gaudot et al., 1994a), and it is different from isoforms detected in nodules (Slezack et al., 1996).

The expression of genes encoding hydroxyproline-rich glycoproteins can also be modulated during AM interactions (Figure 3; see also Franken and Gnädinger, 1994), and in tobacco the level of PR-1 gene transcripts accumulating during AM development is considerably lower than in roots challenged with the fungal pathogen *Chalara elegans* (Gianinazzi-Pearson et al., 1992). Moreover, the localization of defense-related gene expression is very restricted in fully developed mycorrhizal tissues. As previously mentioned, PR-1 protein is detected only in the symbiotic interface between the PAM and the fungal wall (Gianinazzi-Pearson et al., 1992). Transcripts of PAL, CHS, β-1,3-glucanase, chitinase, and PR-1 protein accumulate discretely in arbuscule-containing cells (Harrison and Dixon, 1994; Lambais and Mehdy, 1995; Blee and Anderson, 1996; Gianinazzi-Pearson et al., 1996b), and expression of the *gst1* gene encoding glutathione-S-transferase (as detected by a *gst1* promoter–GUS fusion) is restricted to colonized cortical cells in AM transgenic potato roots (Strittmatter et al., 1996). This spatial pattern of defense gene expression is very different from that reported in plant–pathogen interactions, in which PR proteins accumulate throughout fungal-infected root tissues (Benhamou et al., 1989, 1990; Tahiri-Alaoui et al., 1993b), and defense gene expression is activated in cells other than those containing the pathogen (Schmelzer et al., 1989; Strittmatter et al., 1996).

The uncoordinated, transient, weak, and/or very localized expression of plant defense responses to AM fungi contrasts in many respects to those reported for either compatible or incompatible plant–pathogen interactions, which often differ from each other only in timing and extent (Bowles, 1990; Dixon and Harrison, 1990; Collinge et al., 1994). Whether plant defense responses play any role in plant control over the development of the fungal symbionts is a matter for speculation at this stage (for further discussion, see Gianinazzi-Pearson et al., 1996b). In any event, the AM fungi fail to elicit the full cascade of nonspecific defense responses in host roots. This is not due to an inability to elicit pathogen-like responses because AM fungi induce resistance reactions in roots of the Myc^{-1} pea mutants (see the discussion above). Thus, mechanisms should exist for the local suppression of defense responses to levels compatible with symbiotic interactions. Rejuvenation of colonized plant cells, as occurs with arbuscule development, has been postulated as a strategy to suppress or slow down resistance reactions in the case of biotrophic pathogens (Johal et al., 1995). Such localized effects could explain why AM fungi can develop in plants constitutively overexpressing defense genes (reviewed in Gianinazzi-Pearson et al., 1996b).

Nodulation Events

There are a large number of structural and functional similarities between AM and nodule symbioses. For example, cortical parenchyma cells are the same target for AM development and nodule organogenesis, and a plant-derived membrane borders the physiologically active interface between symbionts in both systems (Mellor, 1989). Moreover, flavonoids, which elicit rhizobial *nod* genes, also enhance hyphal growth of AM fungi (reviewed in Phillips and Tsai, 1992) and probably mediate a Nod factor–induced stimulation of mycorrhizal colonization (Xie et al., 1995). Such similarities, together with the strong link between some of the genes determining nodulation and AM formation in legumes, point to common mechanisms regulating at least part of the interactions in the two symbioses.

Some plant molecules associated with interactions between rhizobia and legumes have been located in AM symbioses. In peas, for example, plant proteins and glycoproteins in the matrix surrounding bacteria in nodule infection threads are present in the host wall material around arbuscule hyphae, and oligosaccharides or glycoconjugates of the plant-derived membrane or interfacial matrix around bacteroids in nodule-infected cells are common to the PAM and arbuscule interface (Gianinazzi-Pearson et al., 1991b, 1996a; Perotto et al., 1994; Gollotte et al., 1995b). Transcripts from mycorrhizal roots of *M. truncatula* hybridize with a probe for the early nodulin gene *ENOD12* (M.J. Harrison, unpublished data), which codes for a proline-rich protein involved in *Rhizobium* infection (Scheres et al., 1990). Using transgenic *M. varia* expressing a *pENOD12*–GUS fusion (Pichon et al., 1994), we have identified the arbuscule-containing cells as the site of gene activation in mycorrhizal roots (V. Gianinazzi-Pearson, E.P. Journet, and D. Barker, unpublished data), suggesting that the encoded protein may be another common component of interactions in the two symbioses.

A small number of late nodulins have been identified among in vitro translation products from AM of soybean. One of these proteins was postulated to be nodulin 26, a protein found in the host membrane around bacteroids in nodules (Wyss et al.,

1990). cDNA sequences with high similarities to genes encoding membrane intrinsic channel proteins, including nodulin 26, have recently been cloned from mycorrhizal roots of parsley, tobacco, and fava bean (Roussel, 1995; P. Franken, H. Roussel, and V. Gianinazzi-Pearson, unpublished data). Activation of the corresponding genes in mycorrhizal roots may reflect the existence of a common transporter system in the symbiotic interface of AM and nodules. Transcripts of another late nodulin gene, *VFLb29*, have been found to be induced de novo in the nodules and AM of fava bean (Frühling, 1995; M. Frühling, A.M. Perlick, H. Roussel, V. Gianinazzi-Pearson, and A. Puhler, manuscript submitted). This gene codes for a leghemoglobin that shares unusually low amino acid sequence identity with other legume leghemoglobins. Studies of its expression pattern within mycorrhizal roots will determine whether, and the extent to which, this protein modulates oxygen supply to the fungal symbiont.

Mycorrhiza-Specific Molecular Responses

The search for specific plant genes mediating symbiotic events in AM formation and functioning is still in its infancy. Recent advances have been made using two strategies: first, analyses of polypeptides for changes in protein biosynthesis; and second, cDNA cloning of genes that are differentially regulated during the symbiosis.

Differential biosynthesis of soluble polypeptides accompanies AM development, and numerous quantitative and qualitative modifications have been observed in early and late stages of plant–fungal interactions in AM (Dumas et al., 1990; Arines et al., 1993; Simoneau et al., 1994; Samra et al., 1996). Proteins that increase and decrease in amount or disappear have been described. Induced polypeptides are most frequent and can represent ~4% of resolved polypeptides (Dumas-Gaudot et al., 1994b; Samra et al., 1996). Although such mycorrhiza-induced polypeptides have been called mycorrhizins, in analogy to nodulins, actinorhizins, and haustorins in other plant–microbe interactions (Verma et al., 1992; Roberts et al., 1993; Seguin and Lalonde, 1993), those of plant origin have not been conclusively identified. Mycorrhiza-resistant pea mutants respond differently when challenged with an AM fungus, the major modification being the disappearance of polypeptides normally present in uninoculated plants (Samra et al., 1996). Hence, the lack of repression of genes that are constitutively expressed in plants may be an essential component of compatible interactions leading to AM establishment (Gianinazzi et al., 1995).

Quantitative and qualitative alterations in gene expression have been confirmed in onion and pea AM by in vitro RNA translation (Garcia-Garrido et al., 1993) and differential RNA display (Martin-Laurent et al., 1995), respectively. The frequency of qualitative modifications in differential display products between mycorrhizal and nonmycorrhizal pea roots is similar to that of polypeptide changes (Martin-Laurent et al., 1996; Samra et al., 1996). cDNA clones corresponding to plant genes that are differentially expressed during early stages of AM development have been isolated by differential RNA display from pea (Martin-Laurent et al., 1996) and screening of cDNA libraries from barley (Murphy et al., 1996). Interestingly, apart from one upregulated sequence from barley that is highly similar to a plant H^+-ATPase gene, sequence similarities have not been found, and the functions of the corresponding genes are as yet unknown. Predictions for secondary protein structure indicate that one of the upregulated genes in pea may encode a putative transmembrane protein (F. Martin-Laurent, D. van Tuinen, E. Dumas-Gaudot, V. Gianinazzi-Pearson, S. Gianinazzi, and P. Franken, manuscript submitted).

Finally, a clone encoding a hexose transporter has been obtained from a cDNA library of AM roots of *M. truncatula* (Harrison, 1996). Expression of the corresponding plant gene, which is normally active in phloem and root tip cells, increases in arbuscule-containing cells or cells adjacent to intercellular hyphae. This does not occur in uncolonized regions of a mycorrhizal root system or during interactions with a mycorrhiza-resistant Myc^- genotype of *M. sativa*, suggesting that the inducing signal operates over a short range and results from the presence of the fungus in the cortex. Such a change in cell-type expression may function to supply sugars to root cells containing the fungus in a functioning symbiosis (Harrison, 1996). These results set the pace for future advances toward identifying plant genes with a role in AM formation and function.

CONCLUSIONS

Significant progress in the analysis of plant systems modulating symbiotic interactions in AM has been made through the application of genetic, ultracytological, biochemical, and molecular biology techniques. Mycorrhiza-defective legume mutants provide direct evidence for specific symbiosis-related gene control and afford the potential for analyzing some host genes and gene functions involved in early and late events. However, the range of mutant phenotypes available is still extremely limited. Expansion of the genetic approach to additional mutations is essential to understand more fully the molecular mechanisms leading to morphological integration and reciprocal functional compatibility between AM symbionts. Moreover, mutagenesis programs, including mycorrhiza-forming nonlegumes such as maize or tomato, should indicate to what extent AM development is determined by processes other than those common to nodulation.

Despite obvious similarities between the infection processes of AM fungi and rhizobia in legumes, important differences also exist. A number of nonnodulating legume genotypes are able to form mycorrhiza (Duc et al., 1989; Wyss et al., 1990; Gianinazzi-Pearson et al., 1991b), and some nodule infection thread components are not synthesized around the fungal symbionts in mycorrhizal roots (Gianinazzi-Pearson et al., 1990; Perotto et al., 1994). Evidence for AM symbioses appears in the fossil record before the evolution of legume species. This

raises the interesting possibility that during evolution, rhizobia may have exploited plant processes involved in symbiotic interactions with arbuscular mycorrhizal fungi and modified them to a completely different purpose, that of nodule development (Gollotte et al., 1995b).

Use of heterologous probes for genes that mediate other plant–microbe interactions has provided much information on cell–cell interactions in AM. This approach has been particularly effective in evaluating the restricted expression of defense responses that is essential for compatibility between plant and fungal symbionts. It has also been helpful in determining the extent to which comparisons between mycorrhizal formation and pathogen responses are valid; for example, do plant susceptibility or fungal compatibility factors exist in AM? Clearly, plant–fungal compatibility is an active phenomenon in AM, because inactivation of a single plant gene, as in the case of the Myc^{-1} pea mutants, results in resistance. Moreover, the fact that AM symbioses are widespread in the plant kingdom makes this symbiosis an interesting model system for dissecting the genetic and molecular bases of biotrophic plant–microbe interactions in general.

Although transport processes between plant and fungal cells are crucial for proper functioning of AM, they represent one of the most poorly understood aspects of the symbiosis. The use of tools such as antibody and nucleic acid probes and promoter–GUS fusions in transgenic plants will accelerate the investigation of the ill-defined area of interface function. In particular, processes regulating PAM H^+-ATPase activity and the potential contribution of mechanisms other than H^+-ATPase–driven transport in metabolite exchange at the PAM need to be explored.

The complex cellular relationship between roots and AM fungi necessitates continuous recognition and signal exchange between both partners. These exchanges affect the regulation of genes whose products participate in the metabolic and structural changes leading to the symbiosis (Gianinazzi et al., 1995). Signal or receptor molecules involved in this dialogue have not been identified. Polysaccharides, hormones, and polyamines are among the candidates for signaling, because they are important components of various developmental processes in plants and are associated with AM development (Table 1; Dannenberg et al., 1992; El Ghachtouli, 1995). The search for plant genes mediating symbiotic events in defined root tissues at specific times has begun. The next few years should see exciting advances toward identifying these plant determinants, the molecules they encode, and the ways they are regulated in AM, the most common root symbiosis in the plant kingdom.

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