Carbon Metabolism and Transport in Arbuscular Mycorrhizas

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Colonization of the land by plants some 400 million years ago was associated with the colonization of their primitive roots by soil-borne filamentous fungi (Nicolson, 1975; Simon et al., 1993; Taylor et al., 1995). Today, 90% to 95% of land plants still maintain some type of mycorrhizal association so that "mycorrhizas, not roots, are the chief organs of nutrient uptake by land plants" (Smith and Read, 1997). Of the several mycorrhizal symbioses, arbuscular mycorrhizas are much the most abundant. These are formed by a very wide variety of host plants (including angiosperms, gymnosperms, pteridophytes, and some mosses, lycopods, and psilotales) and a comparatively small group of aseptate filamentous fungi, the Glomales.

The study of arbuscular mycorrhizal (AM) fungi has fundamental and practical importance. First because in most environments "root biology" is actually "mycorrhizal biology", and second because of the practical importance of AM in fields as diverse as sustainable agriculture, horticulture, reforestation, and ecosystem management (Bethlenfalvay and Schüepp, 1994; Barea and Jeffries, 1995). There have been a number of reviews and at least one excellent text (Smith and Read, 1997) in recent years covering various aspects of the AM symbiosis. These include: development and morphology (Smith and Smith, 1997; Barker et al., 1998; Azcón-Aguilar et al., 1999; Bago, 2000), molecular and cellular biology (Gianinazzi-Pearson et al., 1995; Harrison, 1999), and physiology and nutrient transfer at symbiotic interfaces (Cooper, 1984; Smith and Smith, 1990; Koide and Schreiner, 1992; Azcón-Aguilar and Bago, 1994; George et al., 1995; Jakobsen, 1999). A current resource on mycorrhizal research worldwide is maintained at www.mycorrhiza.ag.utk.edu. Here we review the biochemistry of carbon metabolism and transport in the AM symbiosis. Progress in this area has been significant thanks to the use in recent years of diverse methods including molecular biology, cytology, in vitro AM cultures, microscopy, and nuclear magnetic resonance (Bago et al., 2000).

THE SIGNIFICANCE OF CARBON FLUXES AND METABOLISM IN ARBUSCULAR MYCORRHIZAS

AM fungi derive most, if not all, of their carbon from the host plant (Jennings, 1995). Interest in carbon handling has been spurred because this is a fundamental aspect of the symbiosis, because of the large amounts of carbon handled by the symbiosis, and because of the importance of carbon nutrition in attempts to grow the AM fungus axenically (without host plants). The AM symbiosis usually increases plant biomass and photosynthesis and directs the flow of a significant fraction of the host plant's photoassimilate. Estimates vary, but plants have been shown to direct 4% to 20% more photoassimilate to mycorrhizal root systems (Douds et al., 2000; Graham, 2000). The AM symbiosis therefore determines the flow of huge quantities of carbon worldwide—an estimate of 5 billion tons of carbon annually may be reasonable. The cost-benefit analysis (for review, see Douds et al., 2000) and underlying mechanisms are thus of no small ecological and agricultural importance.

The difficulties of studying an obligate symbiont and the practical goal of mass-producing AM fungal innoculum have motivated considerable efforts over several decades to culture AM fungi axenically. Much of the attention in this (hitherto unsuccessful) endeavor has centered on the search for more or less exotic forms or combinations of carbon substrates (Azcón-Aguilar et al., 1999). Although some of these carbon sources succeeded in somewhat prolonging growth of germinating spores or hyphae, (Hepper, 1984; Bécard and Piché, 1989; Azcón-Aguilar et al., 1999) none induced the fungus to complete its life cycle. Other studies have sought evidence for lesions in carbon metabolic capability that might explain the recalcitrance to axenic culture (Hepper, 1984).

DEVELOPMENT AND STRUCTURES

The life cycle of AM fungi begins when fungal propagules (resting spores, Fig. 1A, or separated intraradical or extraradical hyphae) start to grow. Dur-

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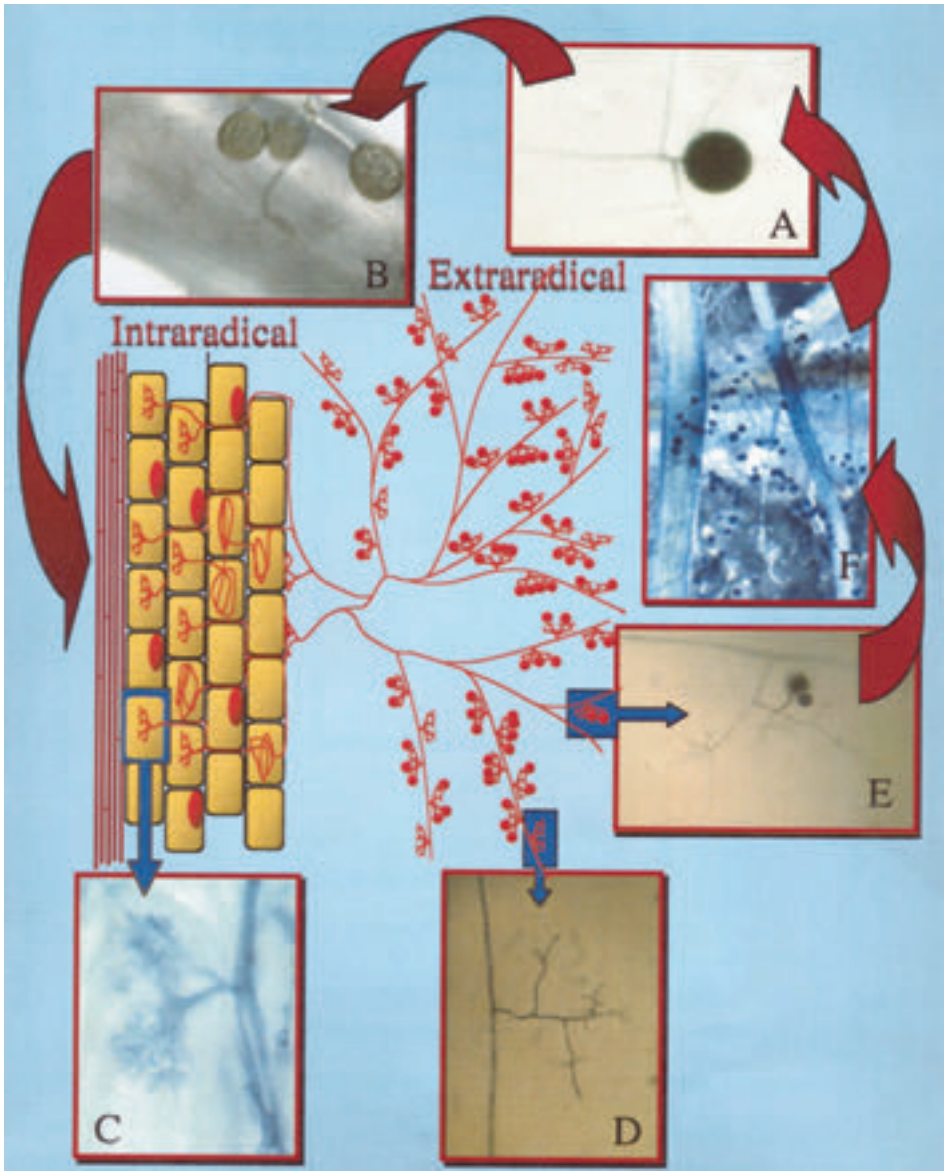

Figure 1. Morphological characteristics relevant to AM fungal metabolism. A, Soil-borne spores have a limited "saprophytic" growth, being unable to complete their life cycle unless they find a host root to colonize. B, After successful colonization, the AM fungus differentiates intraradically forming different structures, the most representative being arbuscules (C). At the same time the fungus profusely develops an extraradical mycelium with different characteristics structures: BAS (D), BAS-spores (E), and resting spores (E and F), thus closing its life cycle.

ing its limited independent growth (for review, see Azcón-Aguilar et al., 1999) triacylglycerides (TAG) and glycogen, the main carbon storage compounds of the fungus, are mobilized (Beilby and Kidby, 1980; Jabaji-Hare, 1988; Bécard et al., 1991; Bonfante et al., 1994; Gaspar et al., 1994, 1997b). This mobilization fuels the development of coenocytic germ tubes and provides carbon skeletons for anabolism, including the de novo synthesis of the chitinous cell wall that surrounds all the fungal structures pictured in Figure 1 (Bonfante-Fasolo and Grippiolo, 1984; Bonfante-Fasolo et al.; 1990; Bago et al., 1996). Asymbiotic growth is maintained for 1 or 2 weeks, during which germ-tube development may reach several centimeters. However if symbiosis is not successfully established within this limited period, AM fungi arrest their growth. Arrest of growth is accompanied by germ-tube septation and nuclear autolysis (Bago et al., 1998c), after which fungal propagules re-enter a state of dormancy and have the ability to regerminate several times (Koske, 1981; Hepper, 1984). Growth arrest before complete depletion of carbon stores may be a strategy to increase the chances of finding an appropriate root to colonize.

If and when the asymbiotically growing AM fungus does contact a host root (Fig. 1B), a series of

signaling events occurs between the partners, which leads to the "acceptance" by the host root of the AM fungus as a symbiont (Giovannetti et al., 1994; Smith and Read, 1997; Blee and Anderson, 2000; Lambais 2000; Shaul et al., 2000). The fungus then develops extensively between and within root exodermal and cortical cells, and forms intraradical structures (for review, see Bonfante-Fasolo, 1984), including arbuscules (Fig. 1C) and lipid-rich vesicles.

Arbuscules consist of hyphae that branch dichotomously and profusely within root cortical cells. Arbuscules and other fungal structures do not penetrate host cell membranes, but invaginate them (Bonfante-Fasolo, 1984). For the arbuscules this results in a huge increase in plant-to-fungal contact surface area, which leads to the assumption that the bidirectional transfer of nutrients in AM probably occurs at the periarbuscular interfaces (Gerdemann, 1968; Harley and Smith, 1983; Bonfante-Fasolo, 1984). This paradigm has been supported by indirect data for the supply of phosphorus to the host by the fungus (Smith and Read, 1997). However, whether arbuscules are the site of root-to-fungus carbon transfer is a matter of debate (Gianinazzi-Pearson et al., 1991; Smith and Read, 1997; Smith and Smith, 1997; Bago, 2000).

Root colonization is accompanied by the development of an extraradical mycelium that includes characteristic branched structures (BAS; Fig. 1D; Mosse and Hepper, 1975; Bago et al., 1998a, 1998b). These may be involved with the uptake of mineral nutrients by extraradical hyphae (Bago et al., 1998b). The external spores develop on some of these BAS (Fig. 1E; Bago et al., 1998b) completing the fungal life cycle. During the AM fungal sporulation phase large numbers of these propagules are formed (Fig. 1F)—as many as 14,000 to 38,000 per root have been estimated in monoxenic cultures (C. Azcón-Aguilar, personal communication; St-Arnaud et al., 1996). Because 45% to 95% of the AM spore carbon pool is neutral lipid (TAG; Beilby, 1983; Jabaji-Hare, 1988; Bécard et al., 1991), these constitute a major sink for the carbon provided by the host plant.

CARBON FLOWS IN THE AM SYMBIOSIS

Figure 2 shows a working model for primary flows of carbon in the AM symbiosis. Emphasis is on uptake, storage, and translocation and on metabolic fluxes for which there is experimental evidence. In the following sections, studies that directly underpin the different features of the model are discussed and remaining questions are reviewed.

Uptake of Carbon by the Fungus

Following the demonstration of significant carbon flow from plant to fungus (Ho and Trappe, 1973; Bevege et al., 1975) arguments from analogy with saprophytic and other symbiotic fungi (Lewis and Harley, 1965a, 1965b; Smith et al., 1969) made sugars strong candidates for the form(s) of carbon transferred. Woolhouse (1975) proposed that host root cortical cells may release sugars to the symbiotic plant-to-fungal interfaces by passive efflux that might be stimulated by the presence of the fungus. Evidence for altered host plasma membrane permeability has been mixed (for review, see Harrison, 1999), and no plant transporters involved in such carbon efflux (Sauer et al., 1994) have yet been iden-

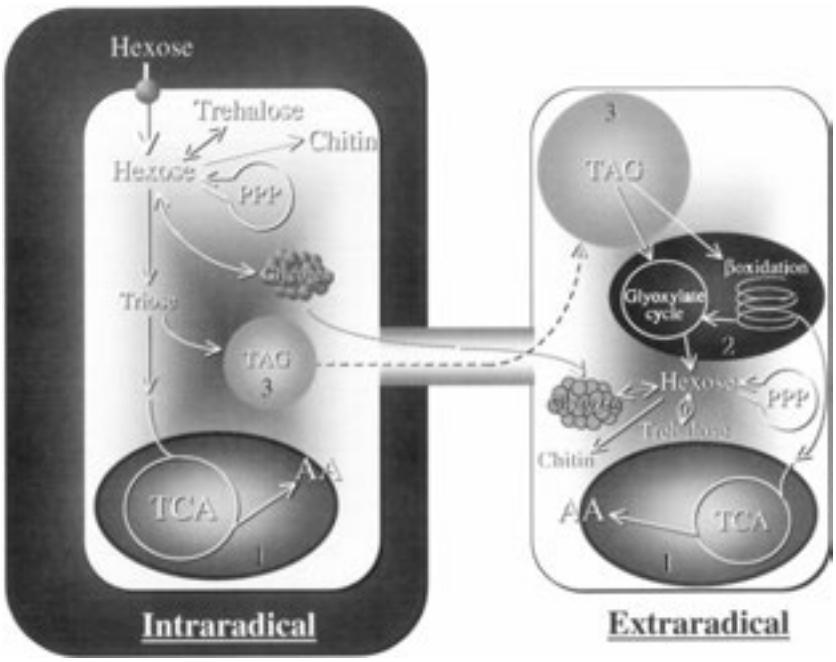

Figure 2. Biochemical pathways of carbon metabolism active in symbiotic intraradical and extraradical AM fungi. Dashed arrows indicate transport events between the different carbon pools. Dotted arrows indicate pathways suggested, but not confirmed to take place. 1, Mitochondria; 2, glyoxysome; 3, lipid bodies.

tified (Harrison, 1999). Studies using isotopic labeling with nuclear magnetic resonance spectroscopy in AM roots (Shachar-Hill et al., 1995) and radiorespirometry measurements on isolated intraradical hyphae (Solaiman and Saito, 1997) have shown that the fungal symbiont can take up and use hexose within the root. In contrast, within the root there is no significant uptake by the extraradical mycelium of Glc, Fru, mannitol, or succinate (Pfeffer et al., 1999).

The finding of modest utilization of Suc by intraradical hyphae (Solaiman and Saito, 1997) raises the possibility that this might also be taken up, though indirect arguments based on fractional enrichments make this less probable in vivo (Shachar-Hill et al., 1995). The presence of elevated levels of host extracellular (acid) invertase activity in diverse biotrophic associations, including AM (Dehne, 1986; Farrar and Lewis, 1987; Snellgrove et al., 1987) is also consistent with hexose as the dominant form taken up. Nonetheless further direct evidence is desirable to conclusively demonstrate whether host Suc is hydrolyzed before uptake. Convincing data from experiments on uptake of, and competition between sugars in a powdery mildew/wheat association has recently allowed Sutton et al. (1999) to show that Suc in that system is indeed hydrolyzed before uptake. Suc utilization of the ectomycorrhizal fungi *Amanita muscaria* and *Hebeloma crustuliniforme* also depends on the cell wall invertase activity of their host spruce (Salzer and Hager, 1991). Similar experiments on an arbuscular mycorrhiza would be valuable.

Molecular characterization of sugar transporters in fungus and host would contribute to the understanding of transferred forms, illuminate the mechanism involved, and open the door to studying regulation of uptake. Fungi can have active and passive sugar transport systems (Blumenthal, 1976; Lagunas, 1993) and there is no direct evidence as to which of these operate in AM fungi. Fungal carbon uptake at the interface might be passive with a concentration gradient maintained by rapid conversion of carbon taken up, as proposed by Bevedge (1975) and consistent with the observed conversion of hexose to trehalose and glycogen (Shachar-Hill et al., 1995). An alternate manner, or together with such a porter, may be active transport. H^+-hexose cotransport by fungi is well known (Sanders, 1988) and such a transporter has been reported in an ectomycorrhizal species (Wiese et al., 2000).

Metabolism in the Mycorrhizal Root

Root carbohydrate pools are substantially altered in AM compared with uncolonized plants (for review, see Douds et al., 2000). For example, starch levels are lower or even absent in arbuscule-containing cortical cells, and labeling in Suc was about four times less when ^{13}C-Glc was provided to colonized roots compared with non-colonized roots (Shachar-Hill et al., 1995). At the level of gene expression, Harrison (1996) demonstrated increased expression of a hexose transporter that is probably involved in uptake in cortical cells near intraradical hyphae (Harrison, 1999). Blee and Anderson (2000) report changes in the expression of invertase in cells containing arbuscules. Respiration in mycorrhizal roots is substantially higher than in non-mycorrhizal ones (Shachar-Hill et al., 1995; Douds et al., 2000; Graham, 2000). These observations are consistent with the transfer of carbohydrate to the fungus and with mycorrhizal roots as a stronger sink for photosynthate than non-mycorrhizal ones (for review, see Douds et al., 2000).

Trehalose and glycogen were found to be the first substantial fungal carbon pool labeled from hexose taken up by the intraradical mycelium (Shachar-Hill et al., 1995). In contrast to observations in the ectomycorrhizal symbiosis (Martin et al., 1985, 1998), the acquired Glc is directly incorporated by the AM fungus into trehalose and glycogen without detectable label scrambling via mannitol or other metabolic turnover. Glycogen and trehalose are both turned over in the intraradical (Shachar-Hill et al., 1995) and in extraradical mycelium and germinating spores (Bago et al., 1999; Pfeffer et al., 1999), which may be associated with buffering cytoplasmic hexose levels. Cytoplasmic hexose is also metabolized via the pentose phosphate pathway (Pfeffer et al., 1999), a finding consistent with the known presence of Glc phosphate dehydrogenase (Saito, 1995). Several glycolytic and tricarboxylic acid cycle enzyme activities have been demonstrated in intraradical hyphae (MacDonald and Lewis, 1978; Saito, 1995). A phosphoglycerate kinase gene of *Glomus mosse* expressed in tomato root systems has recently been characterized (Harrier et al., 1998).

The synthesis of storage lipids is also a substantial sink for carbon in the intraradical hyphae. Lösel and Cooper (1979) showed that the lipid component of mycorrhizal onion roots became labeled when plants photosynthesized in the presence of $^{14}CO_2$, or were supplied with labeled Suc, acetate, or glycerol. TAGs are the most abundant form of lipid in AM fungi (Cooper and Lösel, 1978; Beilby and Kidby, 1980; Nagy et al., 1980; Beilby, 1983; Jabaji-Hare, 1988; Gaspar et al., 1994, 1997a, 1997b). Cis 11,12 hexadecenoic acid is the predominant fungal fatty acid (FA) in all of the *Glomus* species (Graham et al., 1995). Labeling of fungal triacylglycerol from ^{13}C-labeled substrates supplied to the mycorrhizal root suggests that a substantial fraction of hexose taken up is used for lipid synthesis (Pfeffer et al., 1999). The ^{13}C-labeling pattern of the glycerol and FA moieties of the triacylglyceride molecule is consistent with the host-derived hexoses being metabolized (via glycolysis) to triose and acetyl coenzyme A (CoA). After this, FA would be synthesized (via acetyl CoA carboxylase and the FA synthase complex), elongated

and/or desaturated, and finally assembled with a glyceryl moiety via acyltransferases of uncertain subcellular localization (Murphy, 1991).

In oleogenic fungi, the acetyl CoA used for triacylglyceride synthesis is derived from citrate, which, in a biochemical mechanism unique to these type of fungi, is transported from the mitochondrion to the cytosol, then cleaved by a cytosolic citrate lyase (EC 4.1.3.8) to acetyl CoA and oxalacetate (Murphy, 1991). Such a cytoplasmic citrate lyase may also exist in AM fungi.

Translocation by the Fungus

Since hexose is taken up by the fungus within the root and converted to trehalose and glycogen (see above), carbohydrates are obvious candidates to be the form(s) of carbon translocated to the external mycelium (for example, see Gaspar et al., 1997a). TAG in the extraradical structures would then be synthesized from carbohydrate in the extraradical mycelium. However, Pfeffer et al. (1999) concluded from their analysis of ^{2}H labeling of lipids after supplying D_2O to either mycorrhizal roots or to the external mycelium that the FA moieties in the storage TAG of extraradical hyphae had been previously synthesized by the intraradical mycelium and exported. A substantial flux of lipid bodies would therefore be expected along the AM fungal hyphae and there is some support for this; Olsson et al. (1995) observed that the concentration of storage lipids in the extraradical mycelium of an AM fungus decreased relative to phospholipid concentration 1 week after disconnecting the hyphae from the host root, indicating that the neutral lipids were metabolized by the external hyphae, but not replaced. In vivo microscopy indicates that there is indeed a substantial flow of lipid bodies along fungal hyphae (B. Bago, unpublished data). This does not exclude the export of both carbohydrate and lipid, and our recent results on labeling patterns in glycogen after uptake of ^{13}C Glc in the mycorrhizal root suggest that glycogen is also exported to the extraradical mycelium (B. Bago, unpublished data). We are as yet completely ignorant about the regulation of carbon export and indeed about the mechanism of lipid translocation.

Metabolism in the Extraradical Mycelium

Glycolytic enzyme activities assayed in extraradical mycelium have been found to be low or absent (MacDonald and Lewis, 1978; Saito, 1995). Labeling patterns in carbohydrates in the extraradical mycelium when acetate was provided is consistent with a substantial gluconeogenic flux fueled by the glyoxylate cycle (Pfeffer et al., 1999). These two observations are consistent, since the simultaneous operation of glycolysis and gluconeogenesis would represent a futile cycle. The finding that isocitrate lyase and malate synthase are expressed in the extraradical mycelium (J. Jun, unpublished data) provides further evidence that the glyoxylate cycle is active. Since lipid is translocated to the extraradical mycelium, the model of Figure 2 shows the use of this for anabolism in the extraradical mycelium. Lipid is also likely to be the main respiratory substrate since the tricarboxylic acid cycle is active in the external mycelium (MacDonald and Lewis, 1978; Pfeffer et al., 1999; Bago et al., 1999). Since glycolytic activity seems to be low, carbohydrates are probably not a major source of acetyl CoA. Pentose phosphate pathway activity also appears to be substantial in the extraradical mycelium, with enzyme activity measurements (Saito, 1995) and isotopic labeling data (Pfeffer et al., 1999), suggesting higher flux through this pathway than in the intraradical phase.

Implications for Gene Expression

The literature demonstrates and Figure 2 embodies the idea that carbon flows in the AM are organized and highly polarized. Although there is evidence for some heterogeneity among the several thousand nuclei in a spore (Sanders et al., 1995), the genetic pool of the fungus is presumably very largely the same throughout the coenocytic fungus. Therefore the distinct metabolic and transport fluxes in intraradical and extraradical mycelia require that metabolic gene expression must be regulated spatially and temporally to form a "metabolic bipole." Such differential gene expression has been demonstrated for phosphate transporters (Harrison and van Buuren, 1995), but little is known about genes involved in carbon flows. The signals for this differentiation remain unidentified. It is also unknown if such a fungal differentiation is a programmed sequence triggered by one or several signals or whether it requires a continuous input by the plant to be maintained.

Is There a Transfer of Carbon from Fungus to Root in the AM Symbiosis?

Simard et al. (1997) recently reported that there was a net transfer of carbon between plants linked by ectomycorrhizal hyphal networks (see also Finlay and Read, 1986). This result suggests that the direction of carbon transport in mycorrhizas might be reversible. ^{13}C abundance analysis indicates that 0% to 10% (sometimes more) of the carbon of an AM root can be derived from another plant linked to the former by AM fungal hyphae (Watkins et al., 1996; Graves et al., 1997). However, Fitter et al. (1998) found that carbon transported between AM-linked plants remains in the roots of the "recipient" mycorrhizal plant—even when the shoots are removed and allowed to regrow so that carbon flows from roots to the shoots of that plant. This suggests that carbon

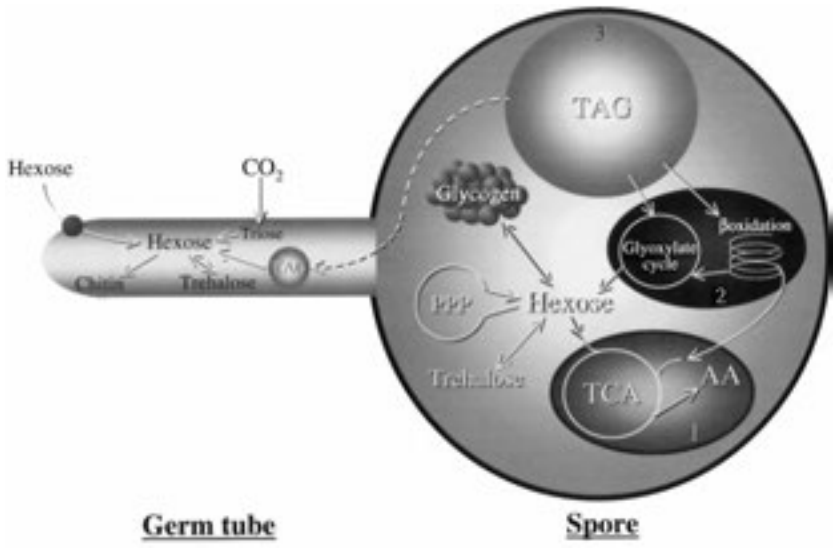

Figure 3. Carbon metabolism in asymbiotic germ-tubes. Dashed arrows indicate transport events between the different carbon pools. Dotted arrows indicate pathways suggested, but not confirmed, to take place. 1, Mitochondria; 2, glyoxysome; 3, lipid bodies.

apparently transferred between connected root systems remains in the fungus (Fitter et al., 1998; Robinson and Fitter, 1999). In accordance with this, these authors propose that the fungus acquires carbon from the root(s) in which it has arbuscules and hyphae, and translocates carbon along extraradical hyphae according to fungal needs, storing the acquired carbon in extraradical spores and older infection units where vesicles are developing. Fitter et al. (1998) found a positive correlation between the amount of carbon transferred and the density of AM fungal vesicles in the roots of the recipient plant, which fits with the export of lipid by the fungus (Fig. 2). Furthermore, supplying labeled permeant substrates (acetate, glycerol, and CO_2) to the extraradical hyphae results in labeling of fungal, but not host carbohydrate (Pfeffer et al., 1999; B. Bago, unpublished data). In accordance with this, we view the model as supporting the conclusion (Robinson and Fitter, 1999) that carbon translocated by the fungus between plant root systems probably does not contribute significantly to host carbon nutrition. However, the transfer of nitrogen from fungus to host can be significant (Frey and Schüepp, 1992; Johansen et al., 1993) and depending on the form transferred, this is likely to entail a significant carbon flux (for example as amino acids) under some conditions.

CARBON FLOWS IN THE ASYMBIOTIC FUNGUS

A model for the metabolism of the asymbiotic AM fungus is shown in Figure 3. This stage of fungal development exhibits characteristics of intraradical and extraradical symbiotic hyphae. As is the case in the extraradical mycelium, germinating spores have substantial gluconeogenic flux mobilizing lipid (TAG) stores to sustain growth (Bago et al., 1999). Enzymatic assays indicate activity of glycolysis (MacDonald and Lewis, 1978; Saito, 1995), the tricarboxylic acid cycle (Hepper et al., 1988; Saito, 1995), and the pentose phosphate pathway (Saito, 1995). Labeling experiments confirm significant carbon fluxes through these pathways and through the glyoxylate cycle, nonphotosynthetic one-carbon metabolism, and the synthesis of Glu and Arg (Bago et al., 1999). Germinating spores also take up hexose, though to a lesser extent than intraradical hyphae. Despite evidence for labeling of lipids from ^{14}C acetate (Beilby, 1983), our ^{13}C labeling experiments indicate that the synthesis of storage FAs in the asymbiotic phase is not a significant flux (Bago et al., 1999). Because TAG is the major carbon storage compound in AM fungal spores, it is tempting to speculate that it is the absence of FA synthesis that prevents the asymbiotic fungus from forming new propagules, making it an obligate symbiont.

FUTURE DIRECTIONS

Figures 2 and 3 illustrate what we know about the main fluxes of carbon; by omission, they also embody our ignorance. Among the topics requiring attention are: (a) completing the identification of metabolic pathways active in the different phases of the fungus, (b) molecular characterization and in situ localization of plant and fungal carbon transporters, and (c) identification of the enzymes governing flux through the different metabolic pathways, and molecular characterization of the fungal genes encoding them. Knowledge in these areas will provide the context and tools for tackling the most important questions about carbon flows in the AM symbiosis—these concern the spatial and temporal regulation of carbon flows by development, signaling, and environment.

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