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Diet of Arbuscular Mycorrhizal Fungi: Bread and Butter?

Mélanie K. Rich,[1,‡] Eva Nouri,[1,‡] Pierre-Emmanuel Courty,[1,2] and Didier Reinhardt[1,*]

Most plants entertain mutualistic interactions known as arbuscular mycorrhiza (AM) with soil fungi (*Glomeromycota*) which provide them with mineral nutrients in exchange for reduced carbon from the plant. Mycorrhizal roots represent strong carbon sinks in which hexoses are transferred from the plant host to the fungus. However, most of the carbon in AM fungi is stored in the form of lipids. The absence of the type I fatty acid synthase (FAS-I) complex from the AM fungal model species *Rhizophagus irregularis* suggests that lipids may also have a role in nutrition of the fungal partner. This hypothesis is supported by the concerted induction of host genes involved in lipid metabolism. We explore the possible roles of lipids in the light of recent literature on AM symbiosis.

Trends

AM fungi receive monosaccharides, in particular glucose, from the plant host.

However, the fact that AM fungi lack FAS-I suggests that AM fungi may also receive lipidic nutrients.

The coordinated induction in the host of genes involved in fatty acid and lipid biosynthesis provides further support to this idea.

Thus, AM fungi may receive a complex diet from their hosts.

Carbohydrates in AM Fungal Nutrition

AM fungi are strictly biotrophic, in other words they depend on their host to complete their life cycle. Although the basis of biotrophy is poorly understood, it has been suggested that it is due to the dependency of AM fungi on some essential nutritional factor(s) from the host [1]. Direct uptake measurements revealed that intraradical mycelium (IRM) can take up carbohydrates, whereas extraradical mycelium (ERM) did not acquire significant amounts of carbohydrates [2,3]. Perhaps this is because AM fungal genes involved in nutrient uptake are expressed only in the host, which would explain their biotrophy. Tracer studies on mycorrhizal roots, and respirometric analysis on isolated intraradical hyphae have established glucose as a central substrate for AM fungi [3–5], and indeed a monosaccharide transporter has been identified in the fungal partner [6]. Mycorrhizal roots represent strong carbon sinks [2,4,7], suggesting that they attract sucrose from photosynthetic source tissues. Sucrose is thought to be cleaved in the vicinity of the fungus by invertases and sucrose synthase [8], resulting in monosaccharides (glucose and fructose) that are released to the fungus and taken up by its IRM [5,6]. However, in the fungal storage compartments – the spores and the vesicles – carbon is stored primarily in the form of lipids, with minor amounts of the glucose polymer glycogen. We discuss here salient issues relating to carbohydrate and lipid metabolism in AM fungi and their significance for fungal nutrition during symbiosis.

Lipid Metabolism in AM Fungi – Open Questions and Surprises

Although the aforementioned carbon fluxes in AM fungi have been firmly established, several questions remain open. AM fungi store and transport most of their carbon in the form of lipids [9–12]. However, AM fungi cannot produce the basic fatty acid (FA) palmitate (C16) in the absence of their host (as shown in two distantly related species, *Rhizophagus irregularis* and *Gigaspora rosea*), while FA elongation and desaturation can occur independently of the plant [13]. This and similar results suggested that AM fungi can only synthesize C16 in the IRM [3,13]. Taken together, the available evidence suggests that AM fungi take up sugars (mainly glucose), then convert them to lipids in the IRM for the export to the ERM (and to the spores), where a significant proportion of the lipids is converted back to carbohydrates by the glyoxylate cycle and gluconeogenesis [14,15]. In addition, glucose is exported from the IRM in the form of

[1]Department of Biology, University of Fribourg, Route Albert-Gockel 3, 1700 Fribourg, Switzerland
[2]Present address: Agroécologie, AgroSupDijon, Centre National de la Recherche Scientifique (CNRS), Institut National de la Recherche Agronomique (INRA), Université de Bourgogne Franche-Comté, 21000 Dijon, France
[‡]These authors contributed equally.

*Correspondence: didier.reinhardt@unifr.ch (D. Reinhardt).

glycogen [14]. It has been pointed out that the conversion of hexose into lipids and back into hexose represents a highly inefficient and rather unusual strategy because nearly 50% of the carbon is lost [14]. This raises the question why AM fungi do not store most or all of their carbon in the form of glycogen, as is the case in other fungi such as yeast and ectomycorrhizal fungi [16,17].

Surprisingly, recent genomic evidence from *R. irregularis* showed that this fungus lacks the cytoplasmic FAS-I complex [18] which in fungi and animals produces the bulk of FAs [19]. FAS-I is also absent from the genome of the distantly related AM fungus *Gigaspora rosea* [20], indicating that the lack of FAS-I may be a common feature of AM fungi. Nevertheless, *R. irregularis* has retained all components of the mitochondrial FAS-II pathway [18,21], which may contribute to lipid biosynthesis. This pathway would then be exclusively active during the intraradical stages in the host. However, the cytoplasmic FAS-I pathway is usually the predominant source of FAs, whereas the role of the mitochondrial FAS-II pathway remains somewhat enigmatic [22,23]. It is therefore unclear whether mitochondrial FAS-II could account for the abundant lipid reserves commonly found in AM fungi.

Induction of Lipid Metabolism in the Host

On the side of the plant host, genetic and transcriptomic analyses have revealed that AM symbiosis involves the coordinated induction of hundreds of AM-related genes [24–33]. Consistent with a role in AM, many of these genes, including their transcriptional activators, are conserved primarily or exclusively in AM-competent plant species [34–36]. Transcript profiling in several diverse host species showed that many of the genes induced in mycorrhizal roots are predicted to function in lipid metabolism (Table S1 in the supplemental information online), and proteome analysis confirmed that lipid biosynthetic enzymes are indeed induced in cells with arbuscules [37] (Table S1). Because a large part of lipid biosynthesis of plants proceeds in plastids, these findings are consistent with the general activation of plastid proliferation and dynamics in mycorrhizal cells [38].

The activation of FA biosynthesis in colonized cells has been interpreted as a metabolic response to the increased demand for lipids for the periarbuscular membrane [37]. This membrane is continuous with the plasma membrane but represents a specialized membrane domain with symbiosis-specific features, for example phosphate and ammonium transporters [39–41]. Indeed, as a result of the increased surface of the symbiotic interface, the surface area of colonized cells has been estimated to increase up to sevenfold relative to non-colonized cortex cells [42]. This is mainly due to the increasing surface between the host membrane (the periarbuscular membrane) and the finely branched fungal hyphae. Hence, colonization of cortical cells requires the synthesis of an amount of membrane material that corresponds to a multiple of the original plasma membrane. However, several lines of evidence raise questions that point to additional roles of lipids in arbuscular mycorrhiza that are independent of membrane biosynthesis (see Outstanding Questions).

Non-Membrane Lipid Functions?

Membrane lipid biosynthesis is initiated by glycerol-3-phosphate acyltransferase (GPAT) with the ligation of a FA moiety to the *sn1* position of glycerol-3-phosphate [43]. Subsequently, a second FA is attached to the *sn2* position, followed by various additions to the phosphate group (or even its replacement) at the *sn3* position, which will give rise to the polar head group [44,45]. Interestingly, mycorrhizal cells induce a specific GPAT referred to as REQUIRED FOR ARBUSCULAR MYCORRHIZA2 (RAM2) owing to its AM-defective mutant phenotype [46]. Based on its close homology to *AtGPAT6* from *Arabidopsis*, and on the phenotypic complementation of the *ram2* mutant with *AtGPAT6*, RAM2 is predicted to have a special GPAT activity

Box 1. Biosynthesis of the Extracellular Lipid Polyester Cutin

Extracellular lipid polyesters consist primarily of FAs that originate from the plastids [79] (Figure I). The products of FA biosynthesis (C16) are exported from the plastids via the FA transporter FAX1 [80], are activated by long-chain acyl-CoA synthase (LACS) [81,82], and are then imported into the endoplasmic reticulum (ER) by an ATP-binding cassette (ABC) transporter of the A subfamily (ABCA). Transfer of FAs from chloroplasts to the ER can be facilitated by direct contact sites between these two compartments [83]. In the ER, the FAs are further processed by oxidation involving cytochrome P450/CYP enzymes to yield diOH-C16-CoA (and related products) [84], followed by condensation with glycerol-3-phosphate by an *sn2*-specific glycerol-3-phosphate acyl transferase (*sn2*-GPAT) to produce 2-monoacyl glycerol (2-MAG) [85,86]. 2-MAG is exported into the apoplast by ABCG transporters [87]. Finally, the cutin precursors are polymerized on the outer side of the polysaccharide cell wall by cutin synthase (CS), whereby the glycerol is released [78,88]. Proteins (transporters and enzymes) are indicated in blue.

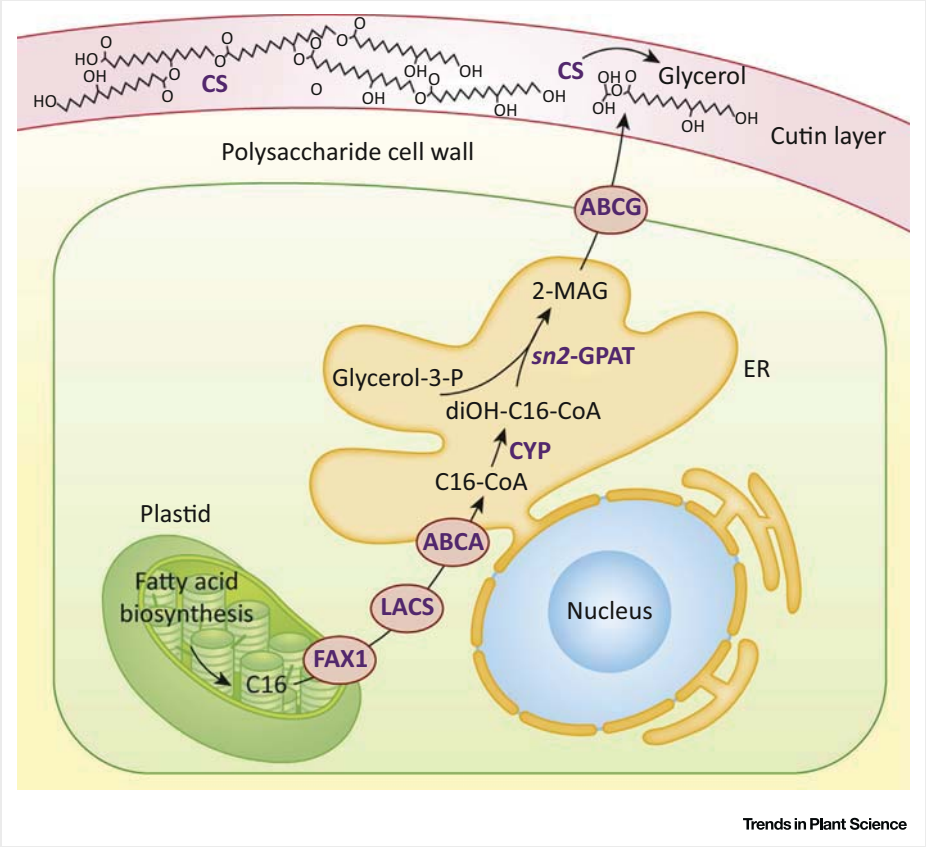

Figure I. Biosynthesis of the Extracellular Lipid Polyester Cutin.

that involves the transfer of FA moieties preferentially onto the *sn*2-position and the simultaneous removal of the phosphate group from the *sn*3-position (phosphatase activity). The products of this reaction, known as *sn*2-monoacyl glycerols (*sn*2-MAGs), cannot be used for membrane lipid biosynthesis, but instead serve as precursors for cutin biosynthesis (Box 1) – protective apoplastic cell wall layers that consist of polymerized hydroxy FAs and dicarboxylic acids [47]. RAM2 has been implicated in early signaling at a stage before physical contact between the symbiotic partners [46]. However, the fact that the *RAM2* promoter is strongly induced in cells with arbuscules [48] points to a function of RAM2 in established mycorrhizal cells. What might then be the role of cutin precursors in cells with arbuscules?

Intriguingly, mycorrhizal roots induce a pair of half-size ATP-binding cassette (ABC) transporters of the G family (ABCG) that are required for AM. Their mutation results in defects in

arbuscule morphogenesis, hence their names STUNTED ARBUSCULE (STR) and STR2 [49,50]. Interestingly, STR and STR2 are closely related to exporters of the lipidic precursors of cutin, suberin, and pollen surface polymers (sporopollenin) (Figure 1) [51–62]. Although the non-mycorrhizal species *Arabidopsis thaliana* has over 20 ABCGs, it lacks close homologs (potential orthologs) of STR and STR2, in contrast to AM-competent monocots and dicots [50]

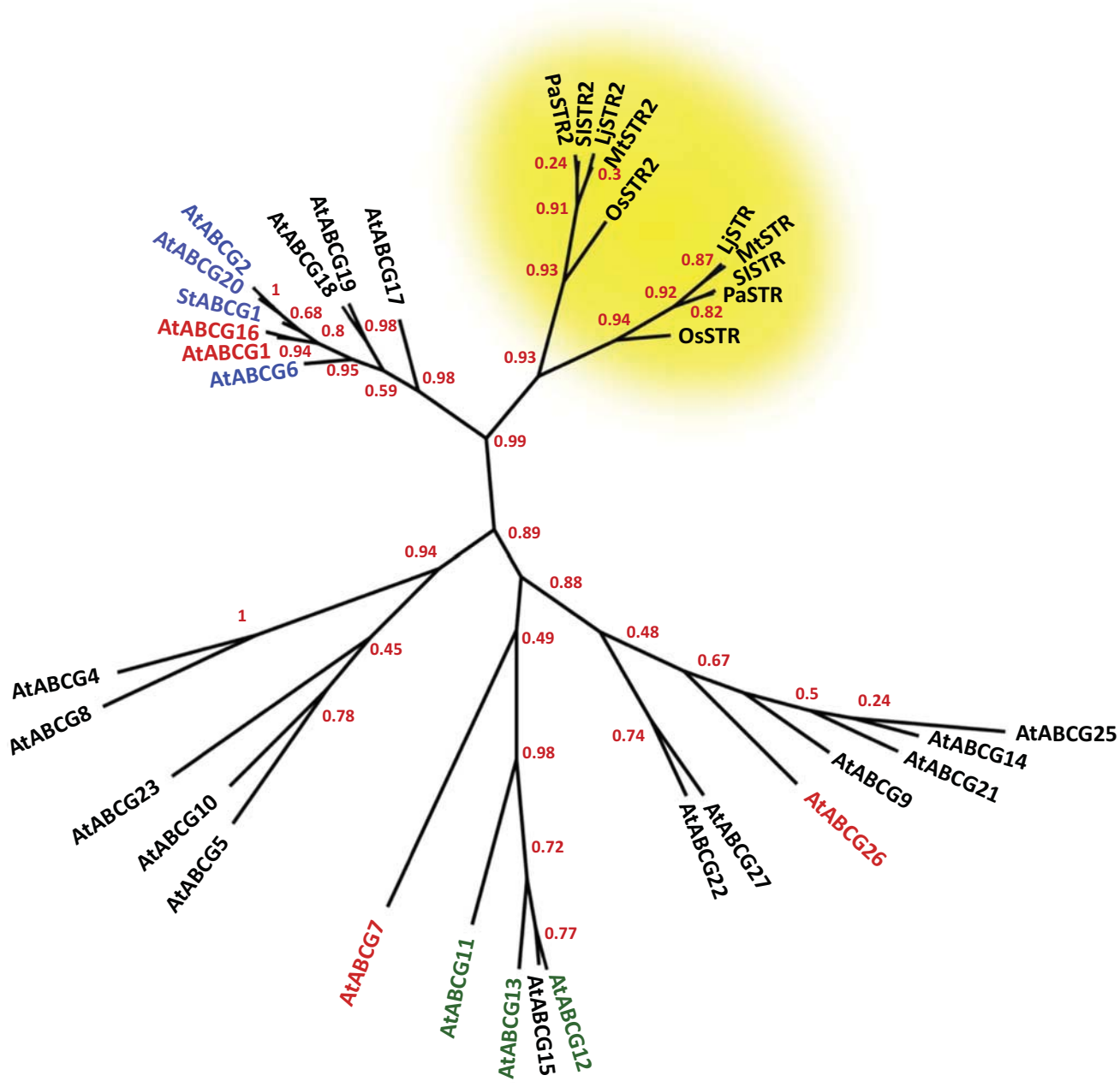

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Figure 1. Phylogram of the ABCG Family of *Arabidopsis thaliana* and the Arbuscular Mycorrhiza (AM)-Related STR and STR2. The entire subfamily of *A. thaliana* (At) ATP-binding cassette (ABC) transporters of type G (ABCG), and a suberin-related ABCG from *Solanum tuberosum* (St), was clustered together with STUNTED ARBUSCULE (STR) and STR2 from *Medicago truncatula* (Mt) and *Oryza sativa* (Os), as well as their closest homologs from *Lotus japonicus* (Lj), *Petunia axillaris* (Pa), and *Solanum lycopersicum* (Sl). Functionally characterized *Arabidopsis* ABCGs are highlighted according to their role in the transport of precursors for cutin (green), suberin (blue), or the exine pollen wall (red). The STR/STR2 branch is highlighted in yellow. For comparison see STR phylogenies in [49,50].

(Figure 1). Based on these findings, we hypothesize that AM-competent plant species have evolved an inducible pathway for lipid secretion that has been lost in *Arabidopsis*.

STR and STR2 are localized to the periarbuscular membrane where they may secrete an unknown substance (potentially MAGs) into the symbiotic interface [46,50]. Cutin is a hydrophobic polymer that limits transpiration and the diffusion of aqueous solutes [47], and therefore would be expected to interfere with nutrient exchange. Indeed, despite extensive microscopic analysis, such polymers have not been detected in cells with arbuscules [63,64]. What could then be the function of secreted MAGs in AM?

Lipid Transfer from the Host Plant to the AM Fungus?

In the absence of a visible extracellular lipid polymer, it is tempting to speculate that the induced lipid-related pathway in the plant host may be linked to the unusual lipid metabolism of AM fungi. For example, secreted lipids from the plant may represent an essential nutritional resource for AM fungi (Figure 2). Given the absence of the FAS-I complex from AM fungi, and the fact that lipids are the main storage form of carbon in AM fungi, the transfer of lipids from the plant to the fungus would circumvent the metabolically inefficient conversion of sugars to FAs through glycolysis, pyruvate decarboxylation, and the mitochondrial FAS-II pathway in the IRM of the fungus. However, Trépanier and colleagues have refuted the transfer of C16 from the plant to the fungus based on the fact that the relative labeling of fungal FAs (vs plant FAs) was >10-fold higher when mycorrhizal roots were supplemented with labeled sucrose versus when they received labeled acetate [13]. Because the conversion of sucrose to FAs in the plant would require a transition through acetyl-CoA, it would be expected that a similar level of labeling should be generated with both sucrose and acetate if the FAs were produced in the plant and transferred to the fungus.

However, considering the strong sink function of mycorrhizal colonies in the root cortex, an alternative scenario could account for the high labeling of fungal FAs from sucrose. The preferential transfer of labeled sucrose to colonized cells (sink function), the concerted induction of the FA biosynthetic machinery, and the specific induction of RAM2 and STR/STR2 may result in a stronger flux of label into fungal FAs than into host FAs. By contrast, labeling from acetate, which diffuses passively through membranes and therefore is not influenced by source–sink relationships, would preferentially label host lipids, because of the large surface of the root and the better accessibility of non-colonized cells. Hence, the available labeling data cannot exclude transfer of lipids from the host to the AM fungus.

Recently, lipidomics analysis of mutants affected in RAM2 and the acyl-ACP thioesterase FatM in *M. truncatula* have provided evidence that mycorrhizal roots exhibit an increased biosynthetic flux toward the C16 FA palmitate, and that they produce the corresponding *sn2*-MAG in a FatM- and RAM2-dependent manner [65]. Although mutants in the ABCG transporter STR did not accumulate the predicted transport substrate (*sn2*-monoacylpalmitate), possibly reflecting tight negative feedback regulation of the biosynthetic pathway, the collective evidence is compatible with a role of the lipid pathway in providing an essential substrate to the AM fungus. However, it remains to be seen whether mycorrhizal host cells can indeed release *sn2*-MAG, and whether such an activity would involve STR/STR2.

The Missing Link: Lipid Uptake by the Fungus

An open question is how lipids secreted by the host could be taken up by the fungus. MAGs are relatively large molecules with low solubility in aqueous environments. In this case, comparison with lipid absorption in the small intestine may be inspiring. Dietary lipids are cleaved in the intestine by lipases to generate free FAs and MAG [66]. These cleavage products are incorporated into micelles together with bile acids and other lipidic molecules [67]. It has been a matter

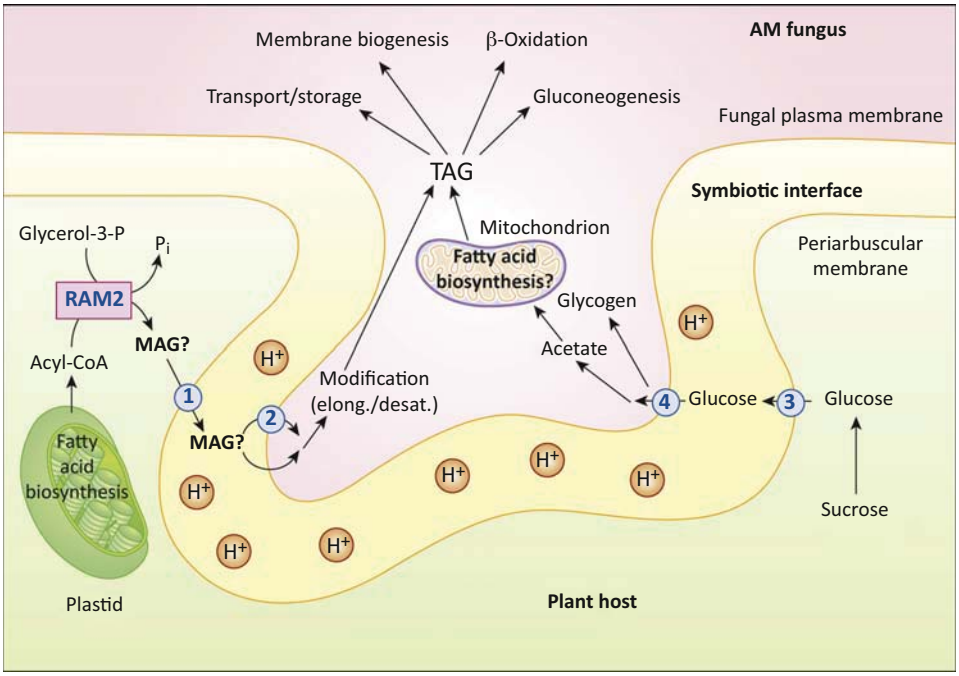

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Figure 2. Hypothetical Lipid and Carbohydrate Metabolism in Root Cortex Cells with Arbuscules. Hypothetical function of the arbuscular mycorrhiza (AM)-induced lipid pathway in a cortex cell of the host plant (green) colonized by an AM fungus (red). Fatty acids (FAs) produced in the plastids (dark green) are transformed into monoacyl glycerols (MAGs) that are secreted into the periarbuscular space (light brown) by ABCG transporters (1). The periarbuscular space represents an acidified compartment (indicated by H^+), from which the secreted MAGs could be taken up by the fungus (intact or cleaved) by active uptake (2) or by passive diffusion (adjacent arrow) as a source of reduced carbon and used for the biosynthesis of triacylglycerol (TAG) or other lipids. This pathway would be active in parallel to the carbohydrate-based route that involves the secretion of glucose (3) into the periarbuscular space followed by uptake into the fungal cytoplasm (4) and lipid biosynthesis by mitochondrial FA synthase (FAS-II). In addition, glucose can be polymerized to glycogen for transport and storage. Hypothetical elements such as the hypothetical MAGs, and FA biosynthesis in mitochondria of the fungus, are represented in bold and highlighted with question marks. Proteins (transporters and enzymes) are indicated in purple. Abbreviations, desat., desaturation; elong, elongation; P_i, inorganic phosphate.

of debate to which degree lipid uptake into the enterocytes of the small intestine involves proteins such as lipid-binding proteins (LBPs) or transport proteins [68,69]. However, it is clear that spontaneous diffusible transfer of FAs and MAG into the plasma membrane represents a significant step in lipid uptake [70].

How does this scenario relate to the situation in mycorrhizal roots? The highly branched arbuscules appear ideally suited for lipid absorption, in particular, because the symbiotic interface is extremely narrow and essentially comprises only a thin fungal cell wall between the membranes of the two partners [71]. The periarbuscular space is acidified by H^+-ATPases which energize mineral nutrient uptake by the plant [72–75]. At the same time, the low pH would lead to protonation of free FAs, which have a p*K*a in an aqueous environment of ~4.8 [76]. Protonated FAs are uncharged and therefore can spontaneously diffuse into membranes. This mechanism is thought to facilitate the uptake of free FAs by the intestine [70]. Whether *sn2*-MAGs can be directly taken up and used by AM fungi, or whether they are first cleaved either by lipases from the plant host (Table S1) or AM fungal lipases, remains to be investigated.

The Bread-and-Butter Hypothesis

The capability to synthesize and secrete lipidic substances evolved in early land plants with the need for a protective coating against transpiration, the cutin layer [77,78]. Conceivably, AM-competent plant species evolved a parallel cutin-related pathway (involving RAM2 and STR/STR2) which produced cutin-like monomers that were used by early AM fungi as a substrate in addition to exuded sugars. This situation may have become genetically fixed through coevolution with the emergence of an AM-inducible lipid secretion pathway in the host, and the loss of FAS-I in the AM fungus. Thus, we suggest that AM fungi receive not only bread (in the form of carbohydrates) but also butter in the form of lipids. These could be further processed (by elongation and desaturation) and converted to building blocks for the biosynthesis of fungal membrane and storage lipids, or they could be directly used as a source of energy through β-oxidation. Alternatively, they could be transformed to carbohydrates through gluconeogenesis. Future work on plant and fungal metabolism should address these possibilities with genetic manipulation of the involved players and further metabolomic analysis.

Outstanding Questions

What is the basis of the strict biotrophy in AM fungi?

How can AM fungi live without the FAS-I complex?

What is the relevance of the concerted induction in the plant host of genes that are predicted to function in lipid biosynthesis and secretion?

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Supplemental Information

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