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PLANT SCIENCE

Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant

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Plants form beneficial associations with arbuscular mycorrhizal fungi, which facilitate nutrient acquisition from the soil. In return, the fungi receive organic carbon from the plants. The transcription factor RAM1 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 1) is crucial for the induction of a lipid biosynthetic pathway that is expressed in plant cells accommodating fungal arbuscules. Lipids are transferred from the plant to mycorrhizal fungi, which are fatty acid auxotrophs, and this lipid export requires the glycerol-3-phosphate acyltransferase RAM2, a direct target of RAM1. Lipid export is required and sufficient for the induction of a transcription factor RAM1 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 1) is crucial for acquisition from the soil. In return, the fungi receive organic carbon from the plants. The

A major limitation to plant growth is restricted access to nutrients in the soil. To improve nutrient acquisition, most land plants enter a beneficial symbiosis with arbuscular mycorrhizal fungi (I). In return for mineral nutrients, plants deliver fixed carbon to the obligate biotrophic fungus. Nutrient exchange takes place through highly branched hyphal structures called arbuscules that form in the inner cortical cells of the root (I). Accommodating fungal hyphae requires the extensive transcriptional reprogramming of root cells, a process mediated by GRAS-domain transcription factors, including RAM1 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 1), which plays a critical role in supporting the development of the arbuscular mycorrhizal symbiosis (2, 3).

To better understand RAM1 function, we analyzed gene expression in roots of the Medicago truncatula wild type and ram1 mutant at 8, 13, and 27 days postinoculation (dpi) with arbuscular mycorrhizal fungi. Although the quantity of fungal infection structures increased in the wild type over the mycorrhizal time course, no fully developed arbuscules were formed in ram1 roots at any time point tested (Fig. 1A). Of all up-regulated genes in the wild type, 27% were abolished in the ram1 mutant at 8 dpi, with this portion increasing to 50% at 13 dpi and 59% at 27 dpi (Fig. 1B). Of the 1092 genes affected, 768 genes showed no induction in ram1 roots at any time point during mycorrhization (figs. S1 and S2) and are therefore potential targets of RAM1. Many of these gene products are associated with lipid and carbohydrate metabolism (Fig. 1C). In the wild type, including RAM2, a glycerol-3-phosphate acyltransferase (GPAT) directly regulated by RAM1 and required for arbuscule formation (2, 4); FatM, a fatty acyl-acyl carrier protein (ACP) thioesterase that functions in arbuscule development (5); two homologs of Arabidopsis thaliana adenosine triphosphate-binding cassette (ABC) transporters involved in exporting lipid precursors for extracellular cutin, suberin, and wax deposition (fig. S3) (6); and three AP2-domain proteins with homology to A. thaliana WRI (WRINKLED) transcription factors (fig. S4) (7, 8). The RAM1-dependent WRI genes, which we named WRI5a to WRI5c, are restricted to plant species that form arbuscular mycorrhizal associations (fig. S4) (5), and WRI5b is required for arbuscule development (9). In A. thaliana, WRI genes regulate late glycolysis and fatty acid biosynthesis, supplying precursors for triacylglycerol production in seeds and cutin in floral tissues (7, 8). Like AnWRII, we found that WRI5 genes drive increased triacylglycerol production when overexpressed in Nicotiana benthamiana leaves, suggesting a common function with their A. thaliana homologs (fig. S4) (7, 8).

Overexpression of RAM1 leads to autoactivation of selected gene expression (3), and we found that RAM1 is required for the activation of RAM2, FatM, WRI5b, WRI5c, and ABCG3 (Fig. 2B and fig. S5), as well as additional arbuscule-associated genes that do not function in lipid production, such as PT4 and EXO70D (fig. S5) (10, 11). These genes are probably direct targets of RAM1. RAM2 is a member of a class of GPAT enzymes that specifically provide lipid precursors (2-monoacylglycerols) for the synthesis of extracellular lipid polymers, such as cutin (4,12). Enzymatic analysis of RAM2 revealed that this isoform is selective for the C16:0 substrate palmitoyl-coenzyme A (a likely product of FatM) (13, 14) and produces 2-monopalmitin (fig. S6), which accumulates in mycorrhized roots.

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Fig. 1. RAM1 is required for up-regulation of genes involved in lipid biosynthesis and export during development of the arbuscular mycorrhizal symbiosis. (A) Quantification of mycorrhizal infection structures in WT and ram1 roots at 8, 13, and 27 dpi. H, hyphopodia; IH, intraradical hyphae; A, arbuscules; V, vesicles. Statistical comparisons have been made to the wild type. Values are the mean of 12 biological replicates ± SEM (error bars) (Student’s t test: *P < 0.05, **P < 0.001). (B) Proportional Venn diagrams showing the extent of overlap of genes induced in mycorrhized versus nonmycorrhized WT and ram1 roots. Numbers in italics indicate the proportion of genes that were induced in WT but not in ram1 roots relative to the total number of genes induced in WT roots at a specific time point. (C) Heat map showing the changes in expression levels between mycorrhized versus nonmycorrhized roots (depicted as log2 fold changes (FC)) of lipid-related genes that are dependent on RAM1 for their induction during mycorrhizal colonization. For both (B) and (C), a 1.5-fold cutoff was used for fold changes.
(14, 15). Recent studies suggest that arbuscular mycorrhizal fungi lack type I fatty acid synthase, implying that they cannot synthesize C16:0 fatty acids (16–18) despite using triacylglycerol as their major carbon store (19, 20). It appears that accommodation of arbuscular mycorrhizal fungi is associated with the activation of a lipid export pathway involving RAM2 and several other genes expressed in arbusculated cells (Fig. 2A and fig. S5).

We hypothesize that this pathway has a nutritive function, rather than just a signaling function (4), for the fungus, and consistent with this we found that ram2 could be compensated by a “nurse plant” (i.e., a colonized wild-type (WT) plant grown in the same pot, but with a mesh allowing only the fungal hyphae to grow into the ram2 root compartment (Fig. 3 and figs. S7 to S9)). Under these conditions, mycorrhization of the ram2 mutant is significantly improved compared with that of ram2 grown alone (Fig. 3A), and arbuscules develop fully (Fig. 3B and figs. S7 to S9). This suggests a nutritional role for RAM2, as lipids exported to the fungus by WT roots can be translocated to the fungus in ram2 roots via the common hyphal network (19, 20). Triacylglycerol content in ram2 roots also increases ~10-fold with a nurse plant, but ~17-fold fewer lipid droplets are present in hyphae near developed arbuscules, as compared with the wild type (fig. S9). To assess RAM2 function in lipid delivery to the fungus, we supplied [14C]labeled sucrose to ram2 plants colonized with a nurse plant and analyzed fatty acyl groups in triacylglycerol isolated from ram2 roots and extraradical fungal hyphae (fig. S8) and fungal spores in the ram2 compartment (Fig. 3D). Radiolabeling of fungal acyl groups was reduced by a factor of ~100 compared with WT or ram2 complemented roots (Fig. 3D and fig. S8), despite mycorrhization occurring in ram2 with a WT nurse plant (Fig. 3E and figs. S7 to S9). We conclude that RAM2 is required for the delivery of fatty acyl groups to the fungus.

Our work suggests that lipids, in addition to sugars, play a role in carbon transfer between plants and arbuscular mycorrhizal fungi (19–22). To confirm this, we genetically modified metabolism in M. truncatula in two ways that allowed us to trace the source of fatty acyl groups in fungal lipids without affecting the arbuscular mycorrhizal symbiosis. We isolated M. truncatula mutants in plastidic acetyl-coenzyme A synthetase (ACS) (fig. S10) (23), an enzyme that is essential for conversion of acetoacetate to fatty acids (fig. S10) but not required for normal fatty acid biosynthesis using sucrose as a precursor (24). Radiolabeling of fatty acyl groups in root lipids using [14C]acetate was reduced by a factor of ~13 in ace-1 and ace-2 (fig. S10), but the mutants develop normally and are colonized with arbuscular mycorrhizal fungi (figs. S10 and S11). Fungal hyphae outside the root cannot use sugars or acetate for de novo synthesis of fatty acids (20, 21), so we applied the [14C]labeled substrates directly to growth medium of colonized ace mutants and analyzed fatty acyl groups in triacylglycerol isolated from fungal spores. Acetate labeling of these fungal acyl groups was reduced by a factor of ~13 compared with WT or ace complemented roots (Fig. 4A). By contrast, labeling of fungal acyl groups was not reduced when [14C]sucrose was supplied (Fig. 4B), revealing that incorporation of acetate into fungal lipids is dependent on the genetic status of the host plant.

The second approach was to genetically engineer M. truncatula roots to express the Umbellularia californica fatty acyl–ACP thioesterase (UcFatB) that produces lauric acid (C12:0) (fig. S12) (25). Lauryl group abundance is extremely low in mycorrhized WT root lipids (~0.1 mole % (mol %)) (27). Expression of UcFatB in M. truncatula roots
mycorrhizal fungi appear to be fatty acid auxo-
sugar supply to the carbon economy of the fungus
is unknown but is most likely 2-monopalmitin.
The form of lipid transferred by the pathway
wanes in arbusculated cells, but a
expression of this pathway in arbusculated cells, but a
expression (fig. S11). The fact that
was transformed with pUB1:ACS (ACS) or empty vector control
was colonized with arbuscular
fungi in arbusculated cells (Fig. 4D). The essen-
tial roles for RAM2 and FatM in development of the arbuscular mycorrhizal symbiosis (4, 5, 14)
demonstrate the importance of this pathway. We
previously proposed a signaling role for this path-
way, promoting fungal development at the root
surface (4). This hypothesis is challenged by ex-
pression of this pathway in arbusculated cells, but a
nutritive function does not exclude a signaling func-
tion. The form of lipid transferred by the pathway
is unknown but is most likely 2-monopalmitin.
The relative contributions of host plant lipid and sugar supply to the carbon economy of the fungus
are also unknown. However, because arbuscular mycorrhizal fungi appear to be fatty acid auxo-
trophs (16–18) yet use triacylglycerol as their main
mobile carbon store (19), we propose that the lipid
export pathway contributes a substantial amount
of carbon to arbuscular mycorrhizal fungi.

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Food for fungi
A wide variety of plants form symbiotic relationships in their roots with arbuscular mycorrhizal fungi. The fungi channel inorganic and micronutrients from soil to the plant, and the plant supplies the fungi with organic nutrients. Jiang et al. and Luginbuehl et al. found that as part of this exchange, the plant supplies lipids to its symbiotic fungi, thus providing the fungi with a robust source of carbon for their metabolic needs.

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