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PLANT SCIENCE

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

Leonie H. Luginbuehl,[1] Guillaume N. Menard,[2] Smita Kurup,[2] Harrie Van Erp,[2] Guru V. Radhakrishnan,[1] Andrew Breakspear,[1] Giles E. D. Oldroyd,[1]* Peter J. Eastmond[2]*

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 this symbiosis, and we demonstrate that it is required and sufficient 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. Our work shows that in addition to sugars, lipids are a major source of organic carbon delivered to the fungus, and this is necessary for the production of fungal lipids.

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 (*1*). 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 (*1*). 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) (*2*, *3*). 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 and fig. S1), 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 *AtWRI1*, 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* was sufficient 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 *EXO70I* (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

[1]Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK. [2]Department of Plant Sciences, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.
*Corresponding author. Email: giles.oldroyd@jic.ac.uk (G.E.D.O.); peter.eastmond@rothamsted.ac.uk (P.J.E.)

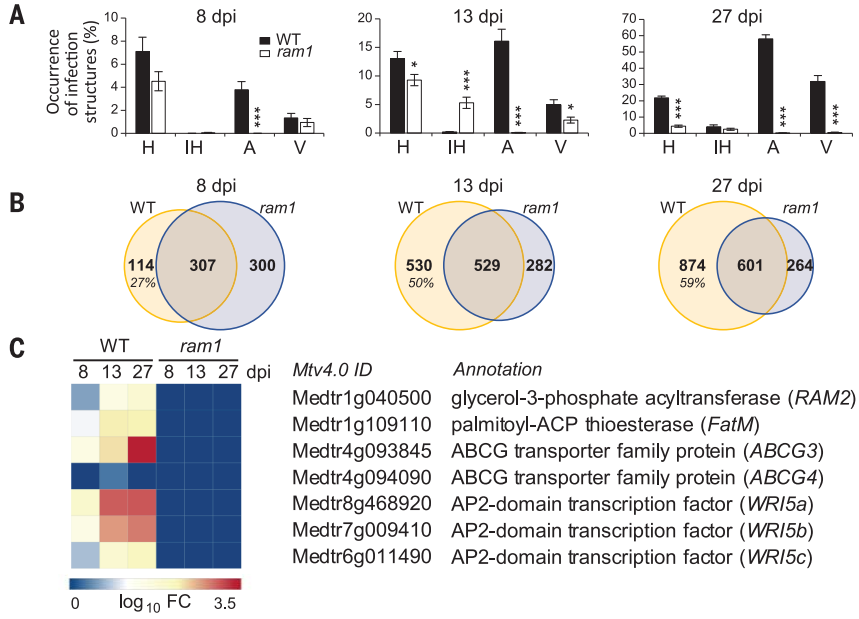

Fig. 1. RAM1 is required for up-regulation of genes involved in lipid biosynthesis and export during development of the arbuscular mycorrhizal symbiosis. (**A**) Quantification of mycorrhizal infection structures in WT and *ram1* roots at 8, 13, and 27 dpi. H, hyphopodia; IH, intraradical hyphae; A, 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 non-mycorrhized 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 $\log_{10}$ 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 ^{14}C-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 acetate 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 [^{14}C]acetate was reduced by a factor of ~13 in *acs-1* and *acs-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 ^{14}C-labeled substrates directly to growth medium of colonized *acs* 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 *acs* complemented roots (Fig. 4A). By contrast, labeling of fungal acyl groups was not reduced when [^{14}C]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*). Lauroyl group abundance is extremely low in mycorrhized WT root lipids [<0.1 mole % (mol %)] (*21*). Expression of *UcFatB* in *M. truncatula* roots

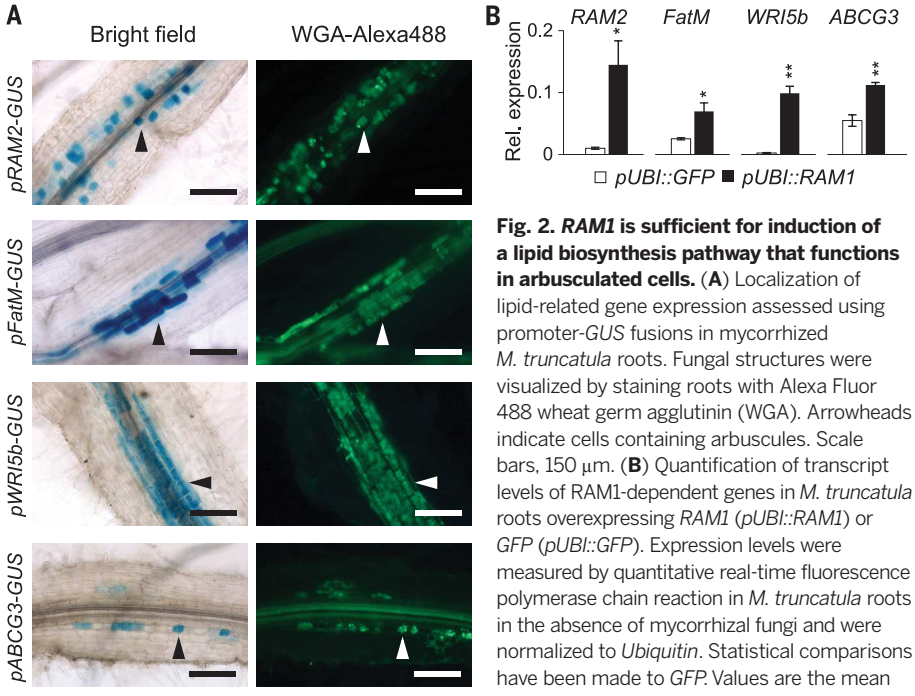

Fig. 2. *RAM1* is sufficient for induction of a lipid biosynthesis pathway that functions in arbusculated cells. (**A**) Localization of lipid-related gene expression assessed using promoter-*GUS* fusions in mycorrhized *M. truncatula* roots. Fungal structures were visualized by staining roots with Alexa Fluor 488 wheat germ agglutinin (WGA). Arrowheads indicate cells containing arbuscules. Scale bars, 150 μm. (**B**) Quantification of transcript levels of RAM1-dependent genes in *M. truncatula* roots overexpressing *RAM1* (*pUBI::RAM1*) or *GFP* (*pUBI::GFP*). Expression levels were measured by quantitative real-time fluorescence polymerase chain reaction in *M. truncatula* roots in the absence of mycorrhizal fungi and were normalized to *Ubiquitin*. Statistical comparisons have been made to *GFP*. Values are the mean of three biological replicates ± SEM (error bars) (Student's *t* test: **P* < 0.05, ***P* < 0.01).

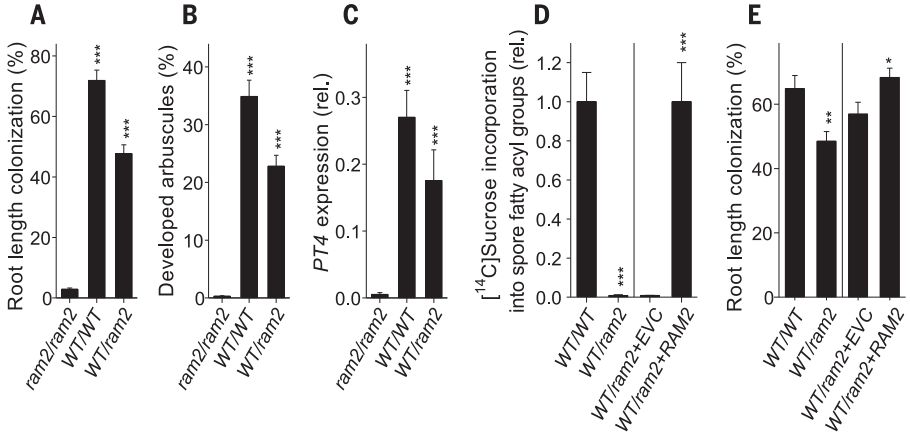

Fig. 3. *RAM2* is essential for the production of fatty acyl groups in fungal lipids. Quantification of (**A**) mycorrhizal colonization, (**B**) developed arbuscules, and (**C**) *PT4* expression (normalized to *EF1*) at 42 dpi in *ram2* and WT test plants (listed second) grown with *ram2* or WT nurse plants. Statistical comparisons were made to *ram2/ram2*. (**D**) Incorporation of [^{14}C]sucrose into fatty acyl groups in triacylglycerol extracted from fungal spores. [^{14}C]Sucrose was infiltrated into the leaves of test plants grown in the presence of a nurse plant at 35 dpi, and spores were analyzed at 77 dpi. Test plants include *ram2*, *ram2* complemented by *pUBI::RAM2* (*ram2* + *RAM2*), or *ram2* transformed with an empty vector control (*ram2* + EVC). Values are expressed relative to the wild type (left) and *ram2* + *RAM2* (right). Values are normalized to one, and corresponding statistical comparisons have been made. (**E**) Level of mycorrhizal colonization in test plants of (D). Values are the mean of 12 biological replicates ± SEM (error bars) [(A) to (C), protected least significant difference (LSD) test: ****P* < 0.001; (D) and (E), Student's *t* test: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001].

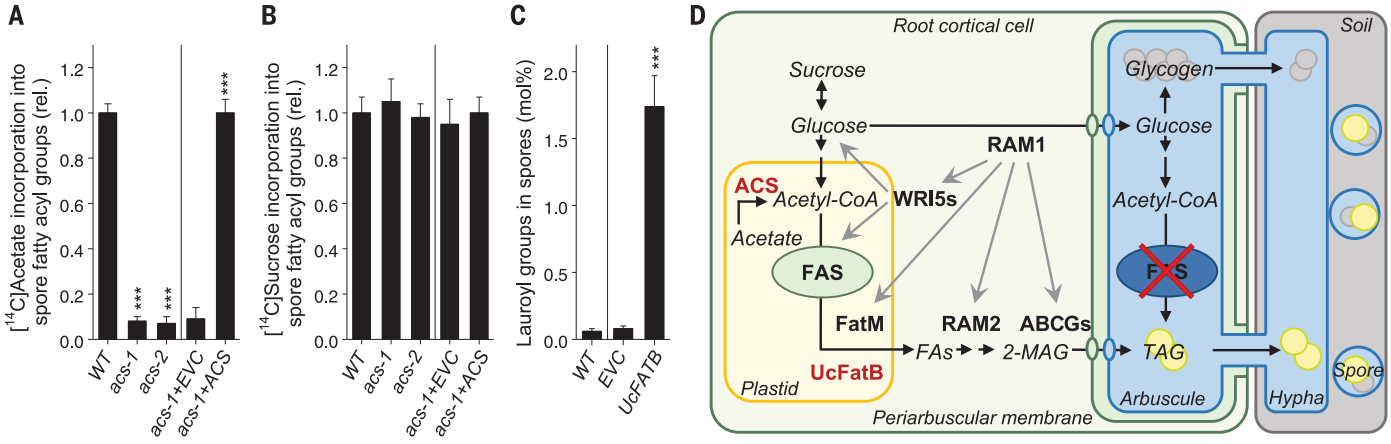

Fig. 4. Fatty acyl groups in arbuscular mycorrhizal fungi are provided by the host. Effect of disruption of plastidic *ACS* on (**A**) [^{14}C]acetate and (**B**) [^{14}C]sucrose labeling of fatty acyl groups in triacylglycerol isolated from fungal spores at 77 dpi, after the radiolabel application to the root compartment at 35 dpi. *acs-1* was transformed with *pUBI::ACS* (*ACS*) or empty vector control (EVC). Values are expressed relative to wild type (left) and *acs-1* + *ACS* (right). Values are normalized to one, and corresponding statistical comparisons have been made. (**C**) Effect of *UcFatB* expression in WT roots on lauroyl (C12:0) groups in triacylglycerol in fungal spores at 77 dpi. Statistical comparisons have been made to EVC. Values are the mean of 12 biological replicates ± SEM (error bars) [(A, left), protected LSD test: ***$P < 0.001$; (A, right) and (C), Student's *t* test: ***$P < 0.001$]. (**D**) Model of the proposed route for biosynthesis of fatty acyl groups stored in fungal triacylglycerols (TAG). FAS, fatty acid synthase; FAs, fatty acids; MAG, monoacylglycerol; CoA, coenzyme A.

led to a ~15-fold increase (~1.5 mol %) of lauroyl groups in root lipids relative to the wild type or empty vector control (fig. S12). When roots expressing *UcFatB* were colonized with arbuscular mycorrhizal fungi, a ~20-fold increase in lauroyl groups was detected in triacylglycerol from fungal spores (Fig. 4C), but root colonization was not affected by *UcFatB* expression (fig. S11). The fact that loss of *ACS* and gain of *UcFatB* in *M. truncatula* leads to the observed changes in radiolabeling and fatty acyl group composition of fungal lipids confirms that fatty acyl groups are delivered from the host plant to arbuscular mycorrhizal fungi.

Our work demonstrates the existence of a RAM1-regulated lipid export pathway that supplies fatty acyl groups to arbuscular mycorrhizal fungi in arbusculated cells (Fig. 4D). The essential 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 pathway, promoting fungal development at the root surface (*4*). This hypothesis is challenged by expression of this pathway in arbusculated cells, but a nutritive function does not exclude a signaling function. 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 auxotrophs (*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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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6343/1175/suppl/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 and S2
References (*26*–*44*)

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

Leonie H. Luginbuehl, Guillaume N. Menard, Smita Kurup, Harrie Van Erp, Guru V. Radhakrishnan, Andrew Breakspear, Giles E. D. Oldroyd and Peter J. Eastmond

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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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