An enzyme regulating triacylglycerol composition is encoded by the *ROD1* gene of *Arabidopsis*

Chaofu Lu[a,b], Zhanguo Xin[a,1], Zhonghai Ren[b], Martine Miquel[a,2], and John Browse[a,3]

[a]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164; and [b]Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717

Edited by Maarten J. Chrispeels, University of California San Diego, La Jolla, CA, and approved September 11, 2009 (received for review August 4, 2009)

The polyunsaturated fatty acids (PUFAs) linoleic acid (18:2) and α-linolenic acid (18:3) in triacylglycerols (TAG) are major factors affecting the quality of plant oils for human health, as well as for biofuels and other renewable applications. These PUFAs are essential fatty acids for animals and plants, but also are the source of unhealthy *trans* fats during the processing of many foodstuffs. PUFAs 18:2 and 18:3 are synthesized in developing seeds by the desaturation of oleic acid (18:1) esterified on the membrane lipid phosphatidylcholine (PC) on the endoplasmic reticulum. The reactions and fluxes involved in this metabolism are incompletely understood, however. Here we show that a previously unrecognized enzyme, phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT), encoded by the *Arabidopsis ROD1* gene, is a major reaction for the transfer of 18:1 into PC for desaturation and also for the reverse transfer of 18:2 and 18:3 into the TAG synthesis pathway. The PDCT enzyme catalyzes transfer of the phosphocholine headgroup from PC to diacylglycerol, and mutation of *rod1* reduces 18:2 and 18:3 accumulation in seed TAG by 40%. Our discovery of PDCT is important for understanding glycerolipid metabolism in plants and other organisms, and provides tools to modify the fatty acid compositions of plant oils for improved nutrition, biofuel, and other purposes.

Arabidopsis | lipid metabolism | oilseeds

Triacylglycerols (TAG) from vegetable oils are a major source of essential fatty acids in the human diet. Linoleic acid (18:2) and α-linolenic acid (18:3) are required for mammalian survival, because these 18- carbon polyunsaturated fatty acids (PUFAs) are precursors in the synthesis of 20- and 22-carbon PUFAs, including arachidonic, eicosapentaenoic, and docosahexaenoic acids, which are important membrane components and substrates for the synthesis of prostaglandins, leukotrienes, and other signaling molecules (1, 2). But *trans* fats produced during the partial hydrogenation of high-PUFA oils are associated with an increasing prevalence of adult and childhood disorders of lipid metabolism, obesity, and related diseases (3, 4). Studies indicate that increasing the proportion of monounsaturated oleic acid (18:1) in vegetable oils provides significant health benefits, as well as improved oxidative stability, which is important both for food uses and for the production of biodiesel and other renewable resources (5, 6). High-oleic, low-PUFA oils are a workable option nutritionally because the requirement for essential fatty acids in the human diet is met with other foods (7, 8). For these reasons, the enzymology and regulation of TAG synthesis and mobilization in both plants and animals remain very active areas of investigation (9–11). The discovery of new enzymes of lipid metabolism in recent years (10, 12, 13) underscores the need to develop comprehensive and correct models of the pathways involved in both mammals and plants that are the major source of dietary essential fatty acids and TAG.

In oil-accumulating cells of plant seeds, 18:1, which is synthesized from acetyl-CoA in the plastids, is desaturated to 18:2 and 18:3 by 2 desaturase enzymes of the endoplasmic reticulum, FAD2 and FAD3 (14, 15). Before desaturation, 18:1 must be incorporated into phosphatidylcholine (PC), the only substrate recognized by the FAD2 and FAD3 desaturases (16). Interestingly, many modified fatty acids, including those with hydroxyl, epoxy, or acetylene groups or conjugated double bonds, also are synthesized on PC by enzymes that likely evolved from ancestral FAD2 proteins (17–19). In current models of seed lipid metabolism, 18:1 is incorporated into PC by 1 of 2 routes. Direct incorporation from 18:1-CoA exported by the plastids occurs, likely through the action of acyl-CoA:*lyso*-phosphatidylcholine acyltransferase (LPCAT) (20, 21). Alternatively, 18:1 may be incorporated into diacylglycerol (DAG) by reactions of the Kennedy pathway, after which 18:1-DAG is converted to PC by CDP-choline:diacylglycerol cholinephosphotransferase (CPT) (22, 23). It has been proposed that the CPT reaction is reversible and provides a mechanism for the production of polyunsaturated DAG for the synthesis of TAGs containing 18:2 and 18:3 (22). Here we present genetic and biochemical evidence for a previously unrecognized enzyme, phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT), that interconverts DAG and PC during TAG synthesis in developing seeds of *Arabidopsis*. PDCT is a gatekeeper enzyme that provides a major route through which 18:1 enters PC for desaturation to 18:2 and 18:3, as well as an important route for the desaturation products, 18:2 and 18:3, to be returned to the DAG pool. Its discovery has important implications for understanding TAG synthesis in seeds and possibly other aspects of lipid metabolism in both plants and animals as well.

Results

Genetic Analysis of the *Arabidopsis rod1* Mutant. In a screen for altered seed fatty acid composition in *Arabidopsis* (24), the *rod1* (*reduced oleate desaturation1*) mutant was identified as having a marked decrease in 18:2 and 18:3 PUFAs and a concomitant increase in 18:1 relative to WT (Table 1). These changes in fatty acid composition were similar to, but smaller than, those observed in the *fad2* mutants (24, 25), raising the possibility that *rod1* represents a hypomorphic allele of *fad2*. Whereas mutations at *fad2* reduced PUFA synthesis in leaves and roots as well as in seeds, significant changes in fatty acid composition were seen only in seeds of *rod1* plants [Table 1; supporting information (SI) Table S1]. Crosses between *rod1* and *fad2* produced F1 seeds with considerably higher PUFA levels than those of either parent (Table 1), confirming that the *rod1* mutation is at a locus distinct from *fad2*.

To determine the genetic basis of the *rod1* mutation, *rod1*

Author contributions: C.L. and J.B. designed research; C.L., Z.X., Z.R., and M.M. performed research; C.L., Z.X., M.M., and J.B. analyzed data; and C.L. and J.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]Present address: Plant Stress and Germplasm Development Research, U.S. Department of Agriculture, Agricultural Research Service, Lubbock, TX 79415.

[2]Present address: Seed Biology, Institute Jean-Pierre Bourgin, INRA Versaille-Grignon, 78026 Versailles, France.

[3]To whom correspondence should be addressed. E-mail: jab@wsu.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0908848106/DCSupplemental.

Table 1. Fatty acid composition in seeds of WT, *rod1*, and *fad2 Arabidopsis* and the crosses between WT × *rod1* and *rod1* × *fad2*

	Mol % of fatty acids in seeds					
	16:0	18:0	18:1	18:2	18:3	20:1
WT	8.4 ± 0.1	3.1 ± 0.2	15.1 ± 0.4	29.2 ± 0.3	19.9 ± 0.3	18.6 ± 0.6
rod1	8.5 ± 0.2	3.3 ± 0.1	32.8 ± 0.6	13.8 ± 0.2	15.6 ± 0.2	20.6 ± 0.1
fad2	6.0 ± 0.1	2.4 ± 0.1	65.0 ± 0.5	0.2 ± 0.1	1.6 ± 0.1	24.0 ± 0.5
WT × *rod1*	8.3 ± 0.5	3.1 ± 0.2	16.9 ± 0.6	29.1 ± 0.9	20.4 ± 0.7	19.9 ± 0.9
rod1 × *fad2*	8.3 ± 0.1	2.4 ± 0.1	20.1 ± 0.2	24.3 ± 0.2	21.0 ± 0.4	22.4 ± 0.2

Data are mean ± SE; $n = 6$.

plants were crossed to Col-0 WT. F1 seeds showed a fatty acid profile similar to that of the WT parent (Table 1). F1 plants were grown and allowed to self-fertilize. Of the 263 F2 plants analyzed, 69 had seed fatty acid profiles similar to that of the original *rod1* seeds (>28% 18:1), while the remaining 194 had fatty acid compositions similar to that of WT (<20% 18:1). This pattern of segregation is a good fit to the hypothesized 3:1 ratio ($\chi^2 = 0.21$; $P > .05$), indicating that *rod1* is a single, recessive Mendelian mutation.

Growth, development, and seed production were very similar in *rod1* plants and WT. In particular, the timing of lipid accumulation in both lines was comparable, a maximum of 7–9 days after pollination. For *rod1*, both the weight of mature seeds (17.7 ± 0.2 μg/seed; average ± SE) and the oil content (4.9 ± 0.3 μg/seed) were indistinguishable from those of WT (17.9 ± 0.1 μg/seed and 4.6 ± 0.2 μg/seed, respectively).

Reduced Radiolabeling of Phosphatidylcholine in *rod1* Seeds. We analyzed the fatty acid compositions of different classes of glycerolipids extracted from seeds during this stage of maximum TAG synthesis. Compared with WT, the *rod1* mutant had substantially lower levels of PUFAs in both TAG and the immediate precursor DAG (Table 2). Surprisingly, however, PC had higher PUFA levels than WT, with the most highly unsaturated fatty acid, 18:3, accounting for 31.7% of total acyl groups, compared to 19.9% in WT (Table 2). The second most-abundant phospholipid in seeds, phosphatidylethanolamine, does not play a major role in TAG synthesis (13); the fatty acid composition of this lipid was similar in the WT and *rod1* samples.

Because PC is the substrate for the FAD2 and FAD3 desaturases that convert 18:1 to 18:2 and 18:3 PUFAs (26), these data indicate the possibility that the *rod1* mutation reduces the transfer of 18:1 into PC for desaturation. Current models of TAG synthesis in oil seeds propose that 18:1 can enter the PC pool through the action of either LPCAT or CPT on 18:1-DAG (20, 21, 23). To gain insight into which of these 2 routes might be blocked in *rod1*, we labeled developing seeds with ^{14}C-glycerol, which predominantly labels the lipid backbone, and ^{14}C-acetate, which labels the acyl groups, in pulse-chase experiments. At the end of the 15-min incubation in ^{14}C-glycerol, WT seeds contained 30% of the total label in PC and 27% of that in DAG, and radioactivity accumulated in TAG during the 3-hour chase (Fig. 1*A*). In contrast, *rod1* seeds contained only 8% of the label in PC but 51% of that in DAG at the end of the pulse; however, radioactivity accumulated in TAG during the chase, as it did in WT. Similarly, the experiment with ^{14}C-acetate revealed reduced incorporation of label into PC at the end of the 15-min labeling pulse in *rod1* compared with WT (Fig. 1*B*). Both sets of data are consistent with *rod1* having a defect that reduces the flux of 18:1 into PC, and additional labeling experiments confirmed this conclusion. The experiment with ^{14}C-glycerol suggests a limitation in the de novo synthesis of PC from DAG, because a lesion in LPCAT would not be expected to restrict the flux of glycerol into PC.

Table 2. Fatty acid compositions of TAG, DAG, PC, and PE isolated from developing seeds of WT and *rod1 Arabidopsis*

	Fatty acid composition (mol %)					
	16:0	18:0	18:1	18:2	18:3	20:1
TAG						
WT	9.2	3.7	17.9	30.5	16.2	18.6
rod1	9.9	3.8	39.1	14.2	12.3	17.9
DAG						
WT	13.2	4.4	14.8	36.0	17.7	6.8
rod1	16.1	5.3	33.8	22.2	10.9	8.6
PC						
WT	17.5	2.4	7.9	45.4	19.9	3.5
rod1	16.1	1.3	6.6	39.8	31.7	1.1
PE						
WT	30.6	3.3	7.5	35.4	18.9	1.3
rod1	33.2	3.2	6.4	34.9	18.3	1.6

Seeds were harvested 9 days after flowering, when TAG accumulation was proceeding rapidly. A repeat analysis yielded similar results.

Identification of the *ROD1* Locus. *Arabidopsis* has 2 genes that encode CPT isozymes, At1g13560 and At3g25585 (27). We determined the sequence of *rod1* genomic DNA at both of these loci, but found no changes from WT. We mapped the *ROD1*

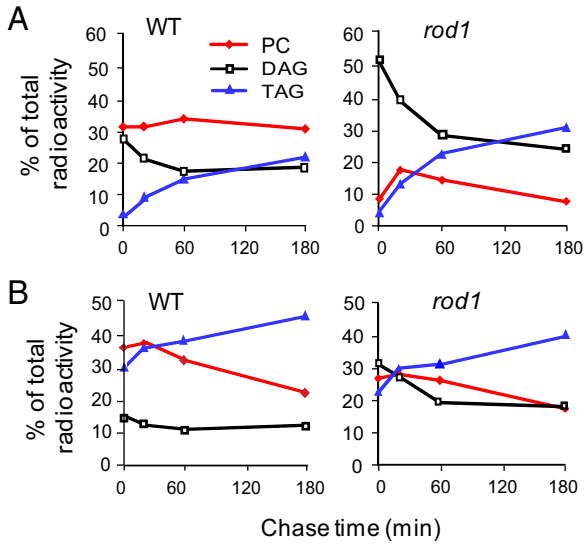

Fig. 1. Lipid synthesis in developing seeds of WT and *rod1* mutant. After a 15-min pulse labeling with [14-C]-labeled glycerol (*A*) or acetate (*B*), the chase was carried out in unlabelled medium. Radioactivities in PC, DAG, and TAG at 0, 30, 60, and 180 min of chase time were determined.

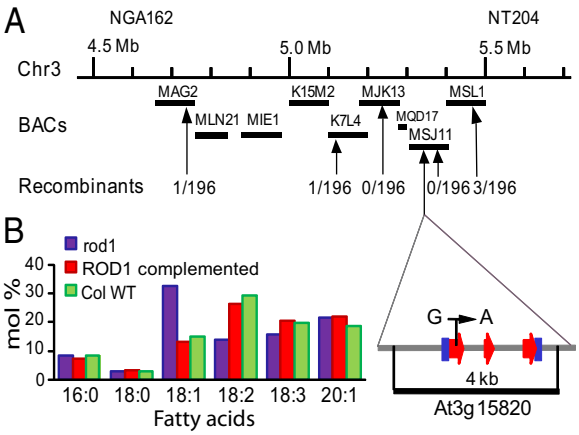

Fig. 2. The *rod1* mutation is in At3g15820. (*A*) The localization and the structure of the *ROD1* gene with the position of the molecular lesion in the mutant. A 4-kb region showing exons (red arrows) and untranslated regions (blue boxes) was used to complement the mutation in *rod1*. (*B*) Comparison of seed fatty acid compositions of the At3g15820 transformants (red bars) and WT (green) indicating that At3g15820 fully restored the *rod1* mutation (purple), thus confirming the identity of *ROD1*.

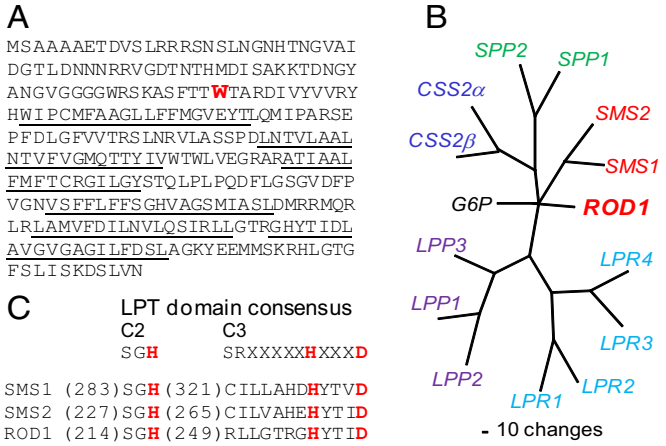

Fig. 3. Sequence analysis of the ROD1 protein. (*A*) Deduced amino acid sequence of At3g15820 with putative transmembrane regions underlined. The site of the W76 stop mutation in *rod1* is shown in red. (*B*) A dendrogram showing the relationship of ROD1 to members of the LPT family in *Homo sapiens* (34). (*C*) Sequences of conserved domains in ROD1 and human SMS proteins showing the catalytic triad in red.

locus, using a population of 800 F2 plants derived from a cross between *rod1* and the Landsberg *erecta* WT (28) (see *SI Text*), to a region of chromosome 3 covered by BAC clones MJK13, MQD17, and MSJ11 (Fig. 2*A*). Within this region, 8 genes were annotated as encoding proteins with known or possible functions in lipid metabolism. After considering the literature (29, 30), we amplified, by PCR, *rod1* genomic DNA corresponding to 6 of these genes, including At3g15820. We identified a G → A transition in this latter gene that is predicted to change Trp^{76} to a stop codon. The remaining 5 genes exhibited no changes from WT.

We invesigated the possibility that this mutation is the basis of the *rod1* lesion and fatty acid phenotype by transforming the mutant with a 4-kb genomic fragment of WT DNA, including the coding region of At3g15820, a total of 2 kb of 5′ and 3′ flanking sequences (Fig. 2*A*), using a vector containing the DsRed transformation marker (31). The fatty acid composition of the transgenic seeds was nearly identical to that of WT (Fig. 2*B*), confirming that At3g15820 is indeed the *ROD1* locus.

The *ROD1* Locus Encodes a PDCT. The protein encoded by At3g15820 has been annotated as a phosphatidic acid phosphatase/(PAP2)-related protein containing Pfam profile PF01569—the PAP2 domain (TAIR 9.0, www.arabidopsis.org). But when we used the ROD1 protein sequence (Fig. 3*A*) to query the Pfam database, the E-value for identification of a PAP2 domain (0.17) was above the recommended cutoff. Only 8 of the 15 most-conserved residues in the Pfam PAP2 profile are present in ROD1. The *Arabidopsis* genome contains at least 4 genes with clearly identified PAP2 domains (E value $<e^{-40}$), including *Lipid-Phosphate Phosphatase1* (*LPP1*; At3g02600) and *LPP2* (At1g15080), which have both been shown to encode lipid-phosphate phosphatase activities (32). ROD1 contains no substantial sequence similarity to these true PAP2 homologues. In addition, we were unable to detect lipid-phosphate phosphatase activity for the ROD1 protein expressed in yeast (*Saccharomyces cerevisiae*) cells (Fig. S1).

When we used the ROD1 amino acid sequence to search the nonredundant protein database using the position-specific iterated BLAST (PSI-BLAST) algorithm (blast.ncbi.nlm.nih.gov), the second iteration identified a mammalian phosphatidylcholine:ceramide cholinephosphotransferase (EC 2.7.8.27). This enzyme, also known as sphingomyelin synthase (SMS), catalyzes the transfer of the phosphocholine headgroup from PC to the alcohol group of ceramide (33). It belongs to the large family of lipid phosphatase/phosphotransferase (LPT) proteins (34). Phylogenetic analysis places ROD1 in close relationship to the SMS1 and SMS2 proteins within the LPT family (Fig. 3*B*), and topology prediction programs identify ROD1 as an integral-membrane protein with up to 6 putative transmembrane domains (Fig. 3*A*), similar to predictions for other LPT proteins (34). In addition, 5 highly conserved residues in the C2 and C3 domains of SMS1, SMS2, and other LPT proteins have been identified at comparable positions in the ROD1 protein (Fig. 3*A* and *C*). Plants do not contain sphingomyelin, but the structure of ceramide is analogous in some respects to that of DAG, prompting us to consider the possibility that ROD1 catalyzes the transfer of phosphocholine from PC to DAG in a reaction analogous to that mediated by SMS in animals. Following biochemical convention, we designate this putative enzyme as phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) in the IUPAC subclass EC 2.7.8.

To directly test for the proposed PDCT activity of the ROD1 protein, we placed the coding sequence of At3g15820 under control of a yeast constitutive promoter in vector p424GPD (35) and expressed the protein in strain HJ091 (*cpt1*::*LEU2 ept1*$^{-}$), which lacks CPT activity (36). We first tested microsomal preparations from HJ091 cells expressing ROD1 and from empty-vector controls for the ability to synthesize PC from DAG and CDP-[^{14}C]choline (36). No activity was detected in either control microsomes or those from cells expressing ROD1 (Fig. 4*A*); however, ^{14}C-labeled PC was produced when ROD1 microsomes were incubated with dioleoyl-[^{14}C]glycerol, and this activity was enhanced in the presence of added PC (Fig. 4*B*). Control microsomes did not exhibit activity in this assay, and ROD1 microsomes that had been boiled before the assay were inactive as well. Because the [^{14}C] radiolabel was in the glycerol moiety of the [^{14}C]-DAG substrate, these assays indicate that ROD1 synthesizes [^{14}C]-PC through transfer of the phosphocholine headgroup from PC to [^{14}C]-DAG. The activity observed in assays without added PC presumably relied on endogenous PC of the yeast microsomes.

Assays with other possible phosphocholine donors indicated that only the phosphocholine headgroup of PC was accessible to

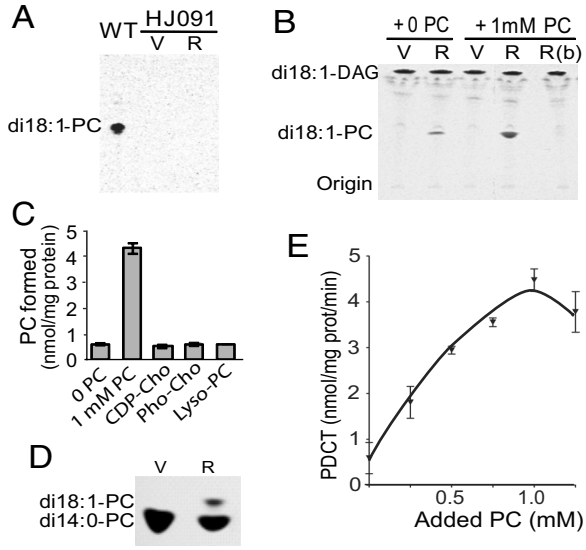

Fig. 4. ROD1 is a phosphatidylcholine:diacylglycerol cholinephosphotransferase. (*A*) Radio-TLC image of CPT assays. (*B*) Radio-TLC image of PDCT assays. Microsomes from DBY746 (WT) or HJ091 *S. cerevisiae* cells transfected with p424GPD (V) or p424ROD1 (R) were incubated with CDP-[^{14}C]choline and diolein for CPT (A) or with [^{14}C-glycerol]di18:1-DAG and PC (0 or 1 mM) for PDCT (B). R(b) indicates boiled p424ROD1 microsomal proteins. (*C*) PDCT assays of microsomes from HJ091 transfected with p424ROD1 incubated with [^{14}C-glycerol]di18:1-DAG and the phosphocholine compounds indicated (at 1 mM concentration). (*D*) Radio-TLC of PDCT assays. Microsomes of HJ091 cells transfected with p424GPD (V) or p424ROD1 (R) were incubated with di14:0-PC [^{14}C-choline] and di18:1-DAG. (*E*) PDCT activity as a function of exogenous PC. Data represent mean and SD of 3 independent reactions.

the ROD1 enzyme. The addition of 1 mM CDP-choline, phosphocholine, or *lyso*-PC did not support [^{14}C]-PC synthesis at rates higher than those of ROD1 microsomes without added PC (Fig. 4*C*). To specifically test for transfer of the PC headgroup, we incubated microsomes with [^{14}C]choline-labeled dimyristoyl-PC and unlabeled dioleoyl-DAG. In this assay, ROD1 microsomes, but not the control, synthesized dioleoyl-[^{14}C]-PC, which separated from the dimyristoyl-[^{14}C]PC substrate on thin layer chromatography (Fig. 4*D*). PDCT activity was highest at pH 6.5–7, linear with time up to 3 min, and linear with protein concentration up to 20 μg of microsomal protein (Fig. S2). Under optimized assay conditions, PDCT activity was 0.6 nmol/min/mg microsomal protein in the absence of added PC, increasing to 4.5 nmol/min/mg with 1 mM added PC (Fig. 4*E*). These results clearly indicate that ROD1 is a PDCT enzyme in *Arabidopsis*.

ROD1-Related Proteins in *Arabidopsis* and Other Organisms. Data from the *Arabidopsis* Gene Expression Atlas (37) indicate that the level of *ROD1* transcript (detected by Affymetrix array element 258249_s_at) is highest in seeds that are accumulating TAG, and that *ROD1* is expressed in other tissues as well (Fig. S3). A related gene, At3g15830, cross-hybridizes to array element 258249_s_at, but data from the *Arabidopsis* Massively Parallel Signature Sequence (MPSS) project (http://mpss.udel.edu/at/) indicate that this gene is expressed only in floral tissues. Our experiments confirmed that At3g15830 is not expressed in seeds (Fig. S4). In addition, no PDCT activity was detected in microsomal preparations from HJ091 yeast cells expressing a cDNA for this gene (see *SI Text*).

Sequences homologous to *Arabidopsis ROD1* are identifiable in many higher plants, including oil crops such as canola (*Brassica napus*) and castor bean (*Ricinus communis*) (Fig. S5), indicating that PDCT likely is an important enzyme of TAG synthesis in many plants. Although no readily identifiable homologues are present in animals, the human *LPT* family contains at least 8 genes that encode proteins of unknown function (34); thus, it remains possible that PDCT will be found to play a role in lipid metabolism in animals as well.

Discussion

We report a recently discovered enzyme, PDCT, that is required for the efficient synthesis of PUFAs during TAG accumulation in seeds. In WT *Arabidopsis*, 49.1% of the fatty acids in seed TAG are polyunsaturated 18:2 and 18:3 (Table 1). These are synthesized from 18:1 on PC of the endoplasmic reticulum by the FAD2 and FAD3 fatty acid desaturases. In the PDCT-defective *rod1* mutant, these fatty acids represent only 29.4% of the total, indicating that 40% (100 × [49.1 - 29.4]/49.1) of the 18:1 that is converted to 18:2 and 18:3 enters PC via the PDCT enzyme.

The potentially large numbers of molecular species and subcellular pools of intermediates in the pathways of seed TAG synthesis (9) make it difficult to represent the relationships comprehensively; however, the simplified scheme shown in Fig. 5 illustrates the role of PDCT and the main fluxes of PUFA-TAG synthesis. For convenience, the fluxes shown in Fig. 5 were calculated for 100 U of 18:2 and 18:3 accumulating in TAG of WT (*Top Right*, box), corresponding to 200 U of total fatty acids from the fatty acid synthase. As shown, 18:1-CoA is the main product exported from the plastid (175 U). Approximately half of this (80 U) is incorporated directly in PC, presumably through the action of LPCAT (21) (reaction 1 in Fig. 5), and much of this (ca. 60 U) is desaturated to 18:2 or 18:3 (reaction 2). In addition, 18:1 is incorporated into DAG by reactions 3–5 (50 U), with most of this transferred into the PC pool by the PDCT enzyme (reaction 6) to be made available for desaturation. The CPT reaction (not shown) is required for de novo PC synthesis, but the

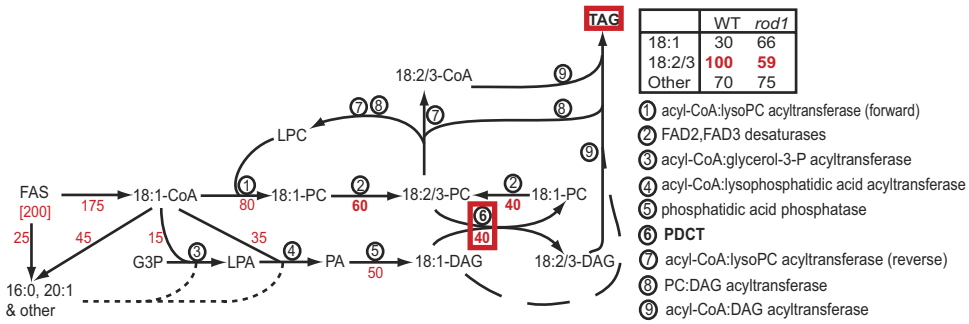

Fig. 5. A simplified scheme of seed TAG synthesis incorporating the PDCT enzyme. The number key lists the enzymatic reactions shown. The fatty acid compositions of WT and *rod1* seeds (*Top Right* and Table 1) were used to calculate the fluxes shown in red for 200 units of fatty acid produced by fatty acid synthase and providing 100 units of PUFA in the TAG.

fatty acid composition of *rod1* seeds (Table 1) and other evidence (9) suggest that the PDCT reaction is responsible for most of the conversion of DAG to PC in developing seeds. Because PDCT is a symmetrical reaction (with 1 DAG molecule generated for each DAG consumed), we assume that 18:2-DAG and 18:3-DAG are produced. This outcome does not necessarily require substrate selectivity of the PDCT enzyme (i.e., 18:2-PC and 18:3-PC over 18:1-PC; 18:1-DAG over 18:2-DAG and 18:3-DAG), because the desaturases will enrich the PC pool with PUFAs. Along with PDCT, 18:2 and 18:3 leave PC through a reverse action of LPCAT (reaction 7) and phosphatidylcholine:DAG acyltransferase (reaction 8), both of which generate *lyso*-PC. The 18:2-CoA and 18:3-CoA produced by reaction 7 are available to aycl-CoA:DAG acyltransferase (reaction 9). In WT seeds, PDCT provides a substantial proportion of the 18:2-DAG and 18:3-DAG substrate. In the *rod1* mutant deficient in PDCT, more 18:1-DAG is incorporated in TAG (dashed line). In both *rod1* and WT, 18:2-CoA and 18:3-CoA can be converted to DAG by reactions 3–5; however, this possibility does not prevent the *rod1* mutation from reducing the accumulation of 18:2 and 18:3 in TAG by 40%.

The high expression of the *ROD1* gene in oil-accumulating seed tissue is consistent with our mutant analysis and enzymology showing that interconversion of PC and DAG by PDCT is an important mechanism for PUFA enrichment of TAG. *ROD1* also is expressed in vegetative tissues (Fig. S3), and PC is a major substrate for 18:1 desaturation in these tissues as well (25). Although the *rod1* mutation does not result in substantial changes in leaf or root fatty acid compositions, PDCT possibly may play a role in lipid homeostasis in vegetative cells of the plant or in remodeling of membrane lipids in response to temperature changes or other environmental perturbations.

PDCT is structurally related to animal SMS and contains the C2 and C3 domains characteristic of the broader LPT family (Fig. 3). These C2 and C3 domains include the histidine and aspartate that are proposed active-site residues involved in phosphate–ester bond cleavage (34). This supports the notion that PDCT uses a catalytic mechanism analogous to that of the SMS enzymes. Further studies are needed to confirm the importance of these residues in PDCT and to investigate whether other aspects of PDCT are analogous to those of the more extensively studied SMS proteins.

PDCT also provides a useful tool for using biotechnology to modify the fatty acid composition of plant oils. For example, because PDCT contributes to the control of PUFA synthesis in seeds, suppression of *ROD1* expression could reduce the need for hydrogenation of oils, along with the attendant production of unhealthy *trans* fats (3, 4). This type of genetic engineering also can allow for the production of biofuels with increased oxidative stability (38) and may reduce the incorporation of saturated fatty acids into membrane lipids. Because PC is also the substrate for enzymes that produce hydroxy-, epoxy-, acetylenic and other modified fatty acids (17–19), our discovery of PDCT provides many opportunities to better understand TAG synthesis in different oil seed species and to improve the fatty acid profiles of vegetable oils for both human health and industrial applications.

Materials and Methods

Genetic Analysis of the *rod1* Mutant. Mutant line *rod1* in the *Arabidopsis thaliana* Col-0 background was isolated from an M3 population after mutagenesis with ethyl methanesulfonate (24). Plants were grown on soil in controlled environment chambers at 22 °C under continuous florescent illumination (150 μmol quanta/m^2/s). Genetic analysis, map-based cloning of the *ROD1* locus, and bioinformatic analyses were performed following conventional approaches (see SI *Materials and Methods*).

Lipid Analysis and Labeling. The overall fatty acid compositions of seeds and other tissues were determined as described previously (31). Pulse-chase labeling was carried out in developing seeds harvested from siliques 9 days after flowering, as described previously (22) (see *SI Materials and Methods*).

ROD1 Enzyme Activity Assays. Growth of HJ091 yeast cells expressing ROD1, preparation of membrane fractions, and assays of CPT were conducted as described previously (39), using 0.1 μmol diolein and 1 nmol [^{14}C]CDP-choline as substrates. Assays of PDCT activity are detailed in *SI Materials and Methods*.

Phylogenetic Analyses. Producing the parsimony bootstrap tree (40) (Fig. 3*B*) involved 1,000 bootstrap replicate data sets, each of which was analyzed using tree bisection reconnection, steepest decent, and other settings to maximize the detection of global optima or maximization of the parsimony optimality criteria.

ACKNOWLEDGMENTS. We thank R. Dewey, C.R. McMaster, and G. Carman for providing strains and advice and M. Schneider, G.-S. Han, M. Lavin, and C. Skidmore for providing technical assistance. This work was supported by grants 2006–35318-17797, 2003–35318-13914, and 2001–345318-10186 from the U.S. Department of Agriculture Cooperative State Research Education and Extension Service, grant DBI-0701919 from the U.S. National Science Foundation, and by the Agricultural Research Center at Washington State University.

1. Funk CD (2001) Prostaglandins and leukotrienes: Advances in eicosanoid biology. *Science* 294:1871–1875.
2. Cunnane SC (2003) Problems with essential fatty acids: Time for a new paradigm? *Prog Lipid Res* 42:544–568.
3. Steinhart H, Rickert R, Winkler K (2003) Trans fatty acids (TFA): Analysis, occurrence, intake and clinical relevance. *Eur J Med Res* 8:358–362.
4. Muoio DM, Newgard CB (2006) Obesity-related derangements in metabolic regulation. *Annu Rev Biochem* 75:367–401.
5. Kinney AJ (1996) Designer oils for better nutrition. *Nat Biotechnol* 14:946.
6. Knothe G (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process Technol* 86:1059–1070.
7. Hunter JE (1990) n-3 fatty acids from vegetable oils. *Am J Clin Nutr* 51:809–814.
8. Williams CM, Burdge G (2006) Long-chain n-3 PUFA: Plant vs. marine sources. *Proc Nutr Soc* 65:42–50.
9. Bates PD, Durrett TP, Ohlrogge JB, Pollard M (2009) Analysis of acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos. *Plant Physiol* 150:55–72.
10. Zimmermann R, et al. (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 306:1383–1386.
11. Guo Y, et al. (2008) Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* 453:657–661.
12. Dahlqvist A, et al. (2000) Phospholipid diacylglycerol acyltransferase: An enzyme that catalyzes the acyl-CoA–independent formation of triacylglycerol in yeast and plants. *Proc Natl Acad Sci USA* 97:6487–6492.
13. Cases S, et al. (2001) Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. *J Biol Chem* 276:38870–38876.
14. Arondel V, et al. (1992) Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. *Science* 258:1353–1355.
15. Okuley J, et al. (1994) *Arabidopsis FAD2* gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. *Plant Cell* 6:147–158.
16. Stymne S, Appelqvist LA (1978) The biosynthesis of linoleate from oleoyl-CoA via oleoyl-phosphatidylcholine in microsomes of developing safflower seeds. *Eur J Biochem* 90:223–229.
17. Lee M, et al. (1998) Identification of non-heme diiron proteins that catalyze triple bond and epoxy group formation. *Science* 280:915–918.
18. Broun P, Shanklin J, Whittle E, Somerville C (1998) Catalytic plasticity of fatty acid modification enzymes underlying chemical diversity of plant lipids. *Science* 282:1315–1317.
19. Cahoon EB, et al. (1999) Biosynthetic origin of conjugated double bonds: Production of fatty acid components of high-value drying oils in transgenic soybean embryos. *Proc Natl Acad Sci USA* 96:12935–12940.
20. Stahl U, Stalberg K, Stymne S, Ronne H (2008) A family of eukaryotic lysophospholipid acyltransferases with broad specificity. *FEBS Lett* 582:305–309.
21. Stymne S, Stobart AK (1984) Evidence for the reversibility of the acyl-CoA:lysophosphatidylcholine acyltransferase in microsomal preparations from developing safflower (*Carthamus tinctorius* L) cotyledons and rat liver. *Biochem J* 223:305–314.
22. Slack CR, Campbell LC, Browse JA, Roughan PG (1983) Some evidence for the reversibility of cholinephosphotransferase-catalyzed reaction in developing linseed cotyledons in vivo. *Biochim Biophys Acta* 754:10–20.
23. Vogel G, Browse J (1996) Cholinephosphotransferase and diacylglycerol acyltransferase: Substrate specificities at a key branch point in seed lipid metabolism. *Plant Physiol* 110:923–931.
24. Lemieux B, Miquel M, Somerville C, Browse J (1990) Mutants of *Arabidopsis* with alterations in seed lipid fatty acid composition. *Theor Appl Genet* 80:234–240.
25. Miquel M, Browse J (1992) *Arabidopsis* mutants deficient in polyunsaturated fatty acid synthesis: Biochemical and genetic characterization of a plant oleoyl- phosphatidylcholine desaturase. *J Biol Chem* 267:1502–1509.

26. Wallis JG, Browse J (2002) Mutants of *Arabidopsis* reveal many roles for membrane lipids. *Prog Lipid Res* 41:254–278.
27. Goode JH, Dewey RE (1999) Characterization of aminoalcoholphosphotransferases from *Arabidopsis thaliana* and soybean. *Plant Physiol Biochem* 37:445–457.
28. Lukowitz W, Gillmor CS, Scheible WR (2000) Positional cloning in *Arabidopsis*: Why it feels good to have a genome initiative working for you. *Plant Physiol* 123:795–805.
29. Fan L, Zheng S, Wang X (1997) Antisense suppression of phospholipase D alpha retards abscisic acid– and ethylene-promoted senescence of postharvest *Arabidopsis* leaves. *Plant Cell* 9:2183–2196.
30. Heilmann I, Mekhedov S, King B, Browse J, Shanklin J (2004) Identification of the *Arabidopsis* palmitoyl-monogalactosyldiacylglycerol delta7-desaturase gene *FAD5*, and effects of plastidial retargeting of *Arabidopsis* desaturases on the *fad5* mutant phenotype. *Plant Physiol* 136:4237–4245.
31. Lu C, Fulda M, Wallis JG, Browse J (2006) A high-throughput screen for genes from castor that boost hydroxy fatty acid accumulation in seed oils of transgenic *Arabidopsis*. *Plant J* 45:847–856.
32. Pierrugues O, et al. (2001) Lipid phosphate phosphatases in *Arabidopsis*: Regulation of the *AtLPP1* gene in response to stress. *J Biol Chem* 276:20300–20308.
33. Huitema K, van den Dikkenberg J, Brouwers JF, Holthuis JC (2004) Identification of a family of animal sphingomyelin synthases. *EMBO J* 23:33–44.
34. Sigal YJ, McDermott MI, Morris AJ (2005) Integral membrane lipid phosphatases/phosphotransferases: Common structure and diverse functions. *Biochem J* 387:281–293.
35. Mumberg D, Muller R, Funk M (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* 156:119–122.
36. Morash SC, McMaster CR, Hjelmstad RH, Bell RM (1994) Studies employing *Saccharomyces cerevisiae cpt1* and *ept1* null mutants implicate the *CPT1* gene in coordinate regulation of phospholipid biosynthesis. *J Biol Chem* 269:28769–28776.
37. Schmid M, et al. (2005) A gene expression map of *Arabidopsis thaliana* development. *Nat Genet* 37:501–506.
38. Kinney AJ, Clemente TE (2005) Modifying soybean oil for enhanced performance in biodiesel blends. *Fuel Process Technol* 86:1137–1147.
39. Hjelmstad R, Bell R (1991) sn-1,2-diacylglycerol choline- and ethanolaminephosphotransferases in *Saccharomyces cerevisiae*: Mixed micellar analysis of the *CPT1* and *EPT1* gene products. *J Biol Chem* 266:4357–4365.
40. Swofford DL (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)* (Sinauer Associates, Sunderland, MA), Version 4.