Seed Storage Oil Mobilization Is Important But Not Essential for Germination or Seedling Establishment in Arabidopsis[1][W]

Amélie A. Kelly, Anne-Laure Quettier[2], Eve Shaw, and Peter J. Eastmond[3]*

School of Life Sciences, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, United Kingdom

Triacylglycerol (TAG) is a major storage reserve in many plant seeds. We previously identified a TAG lipase mutant called *sugar-dependent1* (*sdp1*) that is impaired in TAG hydrolysis following Arabidopsis (*Arabidopsis thaliana*) seed germination (Eastmond, 2006). The aim of this study was to identify additional lipases that account for the residual TAG hydrolysis observed in *sdp1*. Mutants were isolated in three candidate genes (*SDP1-LIKE* [*SDP1L*], *ADIPOSE TRIGLYCERIDE LIPASE-LIKE*, and *COMPARATIVE GENE IDENTIFIER-58-LIKE*). Analysis of double, triple, and quadruple mutants showed that SDP1L is responsible for virtually all of the residual TAG hydrolysis present in *sdp1* seedlings. Oil body membranes purified from *sdp1 sdp1L* seedlings were deficient in TAG lipase activity but could still hydrolyze di- and monoacylglycerol. *SDP1L* is expressed less strongly than *SDP1* in seedlings. However, SDP1L could partially rescue TAG breakdown in *sdp1* seedlings when expressed under the control of the *SDP1* or *35S* promoters and in vitro assays showed that both SDP1 and SDP1L can hydrolyze TAG, in preference to diacylglycerol or monoacylglycerol. Seed germination was slowed in *sdp1 sdp1L* and postgerminative seedling growth was severely retarded. The frequency of seedling establishment was also reduced, but *sdp1 sdp1L* was not seedling lethal under normal laboratory growth conditions. Our data show that together SDP1 and SDP1L account for at least 95% of the rate of TAG hydrolysis in Arabidopsis seeds, and that this hydrolysis is important but not essential for seed germination or seedling establishment.

Storage oil (triacylglycerol [TAG]) breakdown plays an important role in the life cycle of many plants by providing carbon skeletons that support seedling growth immediately following seed germination and enable seedling establishment (Bewley and Black, 1994; Graham, 2008). This metabolic process is initiated by lipases (EC: 3.1.1.3), which catalyze the hydrolysis of TAG to release free fatty acids and glycerol (El-Kouhen et al., 2005; Quettier and Eastmond, 2008; Li-Beisson et al., 2010). We recently employed a forward genetic screen, using the model oilseed plant Arabidopsis (*Arabidopsis thaliana*), to identify a lipase that is responsible for the first hydrolytic attack on the TAG molecule following seed germination (Eastmond, 2006). The *SUGAR-DEPENDENT1* (*SDP1*) gene encodes a protein with a patatin-like acyl-hydrolase domain that can associate with the oil body surface and is capable of hydrolyzing TAG in preference to diacylglycerol (DAG) or monoacylglycerol (MAG; Eastmond, 2006). Unorthodox patatin-like TAG lipases (PTLs) of this type have also been shown to have an analogous function in yeast (*Saccharomyces cerevisiae*), mammals, and insects (Athenstaedt and Daum, 2003, 2005; Zimmermann et al., 2004; Grönke et al., 2005), suggesting that the PTL gene family might play a conserved role in initiating TAG breakdown in the cytosol of all eukaryotic cells.

Arabidopsis *sdp1* mutants are impaired in TAG breakdown following seed germination but they are not completely deficient, suggesting that there is partial molecular redundancy (Eastmond, 2006). The Arabidopsis genome contains three candidate PTL genes that we have previously designated *SDP1*, *SDP1-LIKE* (*SDP1L*), and *ADIPOSE TRIGLYCERIDE LIPASE-LIKE* (*ATGLL*; Eastmond, 2006). SDP1 and SDP1L are most similar to the yeast PTLs TAG LIPASE3 (TGL3), TGL4, and TGL5 (Athenstaedt and Daum, 2003, 2005), while ATGLL is most similar to *Homo sapiens* ATGL and *Drosophila melanogaster* Brummer (Zimmermann et al., 2004; Grönke et al., 2005). The plant kingdom appears to be unusual in that it contains lipases from both the TGL3 and ATGL branches of the PTLs gene family within individual species (Smirnova et al., 2006; Quettier and Eastmond, 2008).

A coactivator protein called COMPARATIVE GENE IDENTIFIER-58 (CGI58) has also been described in

[1] This work was supported by the United Kingdom Biotechnology and Biological Sciences Research Council (grant no. BB/E022197/1).

[2] Present address: Institut des Sciences du Végétal, Centre National de la Recherche Scientifique Unité Propre de Recherche 2355, Avenue de la Terrasse, 91198 Gif-sur-Yvette cedex, France.

[3] Department of Plant Science, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK.

* Corresponding author; e-mail p.j.eastmond@warwick.ac.uk.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Peter J. Eastmond (peter.eastmond@rothamsted.ac.uk).

[W] The online version of this article contains Web-only data.

www.plantphysiol.org/cgi/doi/10.1104/pp.111.181784

mammals, which has been reported to stimulate ATGL activity (Lass et al., 2006). Although CGI-58 itself lacks lipase activity, homologs have recently been identified in yeast (INCREASED COPPER TOLERANCE1 [ICT1]) and Arabidopsis (CGI58-LIKE [CGI58L]) that have been reported to possess TAG lipase and phospholipase lipase activity, in addition to lysophosphatidic acid acyltransferase activity (Ghosh et al., 2008b, 2009). Interestingly, James et al. (2010) have recently shown that an Arabidopsis mutant in this gene is not impaired in seedling growth, but that it does accumulate TAG in its leaves. Finally a number of additional candidate lipases have been proposed to play a role in TAG hydrolysis following seed germination based on their in vitro catalytic activity and/or their pattern of gene expression (Quettier and Eastmond, 2008; Li-Beisson et al., 2010). The aim of this work was to investigate whether *SDP1L*, *ATGLL*, *CGI58L*, or other lipases are also involved in TAG breakdown following Arabidopsis seed germination and to determine what the physiological consequences of a deficiency in this metabolic process are for germination, postgerminative seedling growth, and establishment.

RESULTS

Identification of Putative TAG Lipase Genes and Isolation of Mutants

Studies in yeast, mammals, insects, and plants have recently identified a family of PTLs that are responsible for initiating the hydrolysis of TAG in the cytosol of eukaryotic cells (Athenstaedt and Daum, 2003, 2005; Zimmermann et al., 2004; Grönke et al., 2005; Eastmond, 2006). A lipase-like coactivator protein has also recently been described in mammals, which has been reported to stimulate PTL activity (Lass et al., 2006). Analysis of the Arabidopsis genome has previously lead to the identification of three homologs named SDP1L (At3g57140), ATGLL (At1g33270), and CGI58L (At4g24160), respectively (Eastmond, 2006; Quettier and Eastmond, 2008; Ghosh et al., 2009; James et al., 2010). Analysis of public microarray data available at http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi (Winter et al., 2007) indicates that *SDP1L*, *ATGLL*, and *CGI58L* are all expressed in a variety of wild-type (Columbia-0 [Col-0]) tissues, including seeds that have been imbibed for 24 h. This time point coincides with seed germination and the onset of TAG breakdown (Eastmond, 2006).

To investigate whether any of these candidate lipases account for the residual TAG hydrolysis in *sdp1* seedlings a reverse genetic approach was taken. T-DNA insertion mutants in *SDP1L*, *ATGLL*, and *CGI58L* were identified on the SALK T-DNA Express Web page (http://signal.salk.edu/cgi-bin/tdnaexpress) and were obtained from the relevant Arabidopsis stock centers. The *sdp1L-1* and *sdp1L-2* alleles contain insertions in the 5′-untranslated region and first exon of At3g57140, at positions −260 and +358 bp relative to the translational initiation codon (Fig. 1A). In *atglL* the T-DNA is inserted in the fourth intron of At1g33270 at +1,225 bp and in *cgi58L* in the first exon of At4g24160 at +210 bp (Fig. 1A; James et al., 2010). PCR experiments using genomic DNA confirmed the location of the insertions (Alonso et al., 2003) and PCR experiments using cDNA from homozygous mutant seedlings showed that they all lack wild-type transcript and are therefore likely to be null (Fig. 1B). No obvious phenotypes were observed under normal growth conditions for any of the three mutants (data not shown). Double, triple, and quadruple mutants were created by crossing *sdp1L-1*, *sdp1L-2*, *atglL*, and *cgi58L* into the null *sdp1* T-DNA alleles, *sdp1-4* and *sdp1-5* (Fig. 1A; Eastmond, 2006). In the case of *SDP1L* data are only shown for the *sdp1L-2* allele because it shares a common genetic background with the other mutants (Col-0). However, we confirmed that the Wassilewskija allele *sdp1L-1* behaved the same as *sdp1L-2* as a single mutant and when combined with *sdp1-4* (Supplemental Fig. S1).

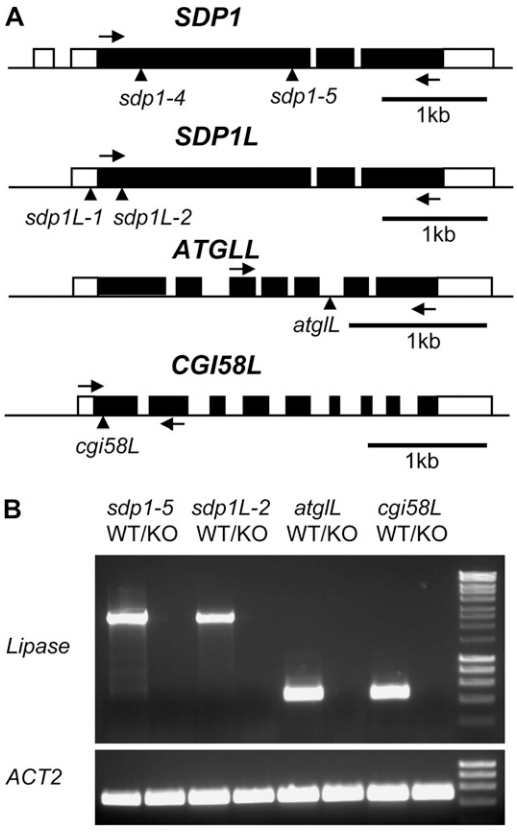

Figure 1. Isolation of T-DNA mutants in SDP1L, ATGLL, and CGI58L. A, Representation of the *SDP1*, *SDP1L*, *ATGLL*, and *CGI58L* loci showing the position of characterized T-DNA insertions. Black bars are exons, and white bars indicate the 5′ and 3′ untranslated regions. Arrows indicate primers used for detection of transcript. B, Detection of transcripts in wild-type (WT) and homozygous knockout (KO) lines *sdp1-5*, *sdp1L-2*, *atglL*, and *cgi58L*. Reverse transcription-PCR was performed on RNA from 2-d-old seedlings. *ACTIN2* was used as a positive control.

SDP1L Is Required for Almost All the Residual TAG Hydrolysis Observed in *sdp1*

To determine whether any of the mutants were impaired in TAG breakdown following seed germination the total fatty acid content and amount of eicosenoic acid (20:1 n-9) were measured in seeds and 5-d-old seedlings grown in the light and in the dark (Fig. 2). 20:1 is almost exclusively found in TAG in Arabidopsis seeds (Lemieux et al., 1990) and therefore has been widely used as a convenient marker for TAG breakdown (Eastmond et al., 2000; Penfield et al., 2004). Care was taken to include both the seedling and the split seed coat, containing the aleurone layer, in each sample since the aleurone has been shown to contain approximately 10% of the total fatty acids in the Arabidopsis seed (Penfield et al., 2004). Of all the lipase single mutants tested, only *sdp1* was significantly impaired in total fatty acid and 20:1 breakdown (Fig. 2). When double-mutant combinations were tested *sdp1-5 sdp1L-2* showed significantly less breakdown of total fatty acids and 20:1 ($P < 0.05$) than *sdp1-5*, while *sdp1-5 atglL* and *sdp1-5 cgi58L* did not (Fig. 2). The decrease in total fatty acid or 20:1 content observed between *sdp1-5 sdp1L-2* seeds and 5-d-old seedlings grown in the dark was typically approximately 10%. Over the same period wild-type seedlings lose >80% of their total fatty acid content and >98% of their 20:1. It has recently been shown that some 20:1 is present in Arabidopsis roots as well as in seeds, albeit at less than 2 mol% of the total fatty acid content (Roudier et al., 2010). Therefore to confirm that the small drop in 20:1 levels observed in 5-d-old *sdp1-5 sdp1L-2* seedlings accurately reflects a fall in TAG content, direct measurements were also performed (Eastmond, 2006). These measurements showed that TAG content declines by 11% (SE ± 3.4, $n = 6$) between seeds and 5-d-old seedlings, which is not significantly different ($P > 0.05$) from the drop detected in 20:1 (Fig. 2B).

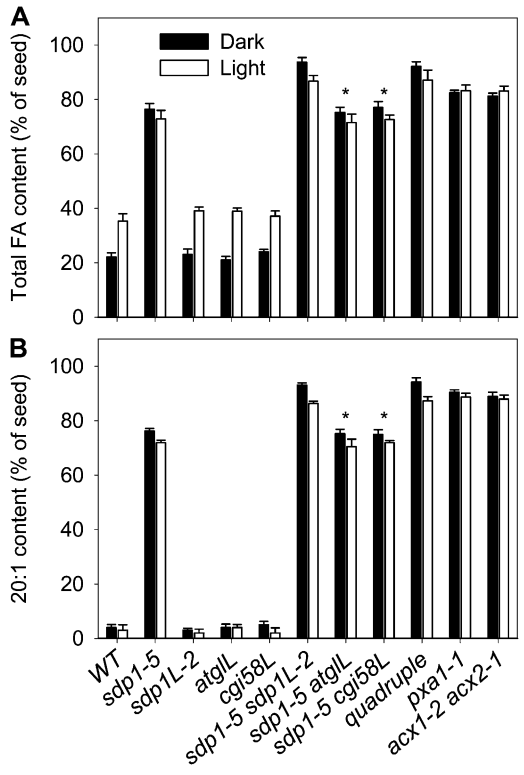

Figure 2. Quantification of fatty acid breakdown during postgerminative growth. Wild-type (Col-0) and mutant seedlings were grown in the dark or the light for 5 d on agar plates containing half-strength Murashige and Skoog salts with 1% (w/v) Suc. Total fatty acid content (A) and eicosenoic acid (20:1) content (B) in the seedlings are expressed as a percentage of the amount present in the same number of seeds prior to germination. Values are the mean ± SE of measurements made on four or more separate batches of 10 to 20 seeds and seedlings plus seed coats. The asterisk denotes that the values do not significantly differ from *sdp1-5* ($P > 0.05$).

For comparison fatty acid breakdown was also measured in the *pxa1-1* and *acx1-2 acx2-1* mutants. *PXA1* (also known as CTS and PED3) encodes an ATP-binding cassette transporter that is required for the import of fatty acyl groups into the peroxisome (Zolman et al., 2001) while *ACX1* and *ACX2* encode long-chain acyl-CoA oxidases involved in fatty acid β-oxidation (Adham et al., 2005). In both *pxa1-1* and *acx1-2 acx2-1* total fatty acid and 20:1 levels fell by 10% to 15% after 5 d (Fig. 2). The severity of the defect in fatty acid breakdown is therefore similar in *sdp1-5 sdp1L-2*, *pxa1-1*, and *acx1-2 acx2-1*. Interestingly, when *sdp1-5 sdp1L-2* seedlings were grown in the light, slightly more total fatty acid and 20:1 breakdown could be detected after 5 d (Fig. 2). A time-course experiment revealed that despite this the maximum rate of 20:1 breakdown was comparatively small in *sdp1-5 sdp1L-2* (Fig. 3). It was equivalent to approximately 5% of the maximum rate in wild type, which occurred between 1 and 3 d after imbibition. To investigate whether ATGLL or CGI58L are responsible for the residual breakdown of fatty acids, measurements were also carried out on seeds and 5-d-old seedlings of triple and quadruple mutants (Fig. 2). However, a small drop in total fatty acid and 20:1 content was still detected in these lines. Measurements performed on *sdp1L-2*, *atglL*, and *cgi58L*, over the course of the first 5 d of postgerminative growth, also suggested that the rates of 20:1 breakdown were not significantly different from wild type in any of these single mutants (Supplemental Fig. S2).

sdp1 sdp1L Is Deficient in Oil Body-Associated TAG Lipase Activity, But Retains DAG and MAG Lipase Activity

We have previously shown that SDP1 associates with the oil body surface in Arabidopsis seedlings and that oil body membranes purified from *sdp1* mutant seedlings have reduced TAG lipase activity (Eastmond, 2006). To investigate whether this activity is further reduced in *sdp1 sdp1L*, TAG lipase assays were performed on oil body membranes from wild type, *sdp1-5*,

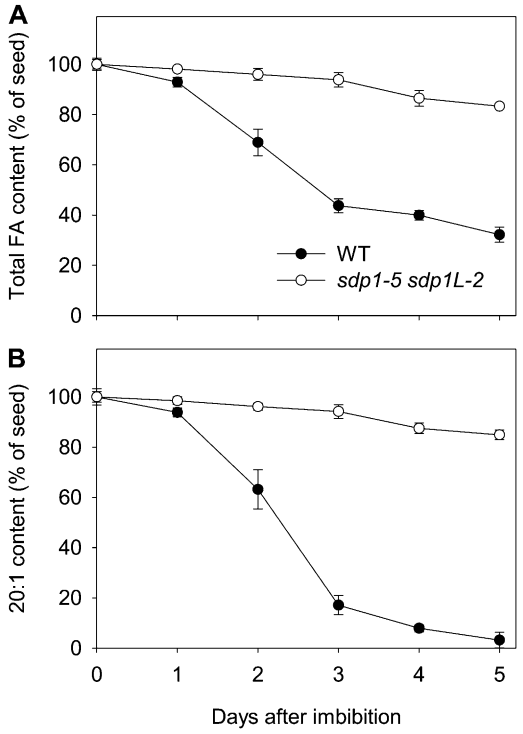

Figure 3. Time course of fatty acid breakdown in wild type (Col-0) and *sdp1-5 sdp1L-2*. A, Total fatty acid content. B, Eicosenoic acid (20:1) content. Seedlings were grown in the light on agar plates containing half-strength Murashige and Skoog salts with 1% (w/v) Suc. Values are expressed as a percentage of the amount found in the same number of seeds prior to germination and are the mean ± SE of measurements made on four or more separate batches of 20 seeds or seedlings plus seed coats.

sdp1L-2, and the double mutant using [^{3}H]triolein as a substrate (Table I). In these experiments TAG lipase activity was reduced by approximately 76% in *sdp1-5* and was not significantly different from zero in *sdp1-5 sdp1L-2* ($P > 0.05$). The possibility that *sdp1-5 sdp1L-2* contains some residual TAG lipase activity cannot be discounted. A post-hoc statistical power calculation suggests that the minimum detectable difference was approximately 9%. There was not a statistically significant difference in TAG lipase activity between wild type and *sdp1L-2* ($P > 0.05$). Lipase assays using [^{3}H]diolein and [^{3}H]monolein as substrates were also performed on oil body membranes and revealed that a substantial amount of DAG and MAG lipase activity remain in *sdp1-5*, *sdp1L-2*, and *sdp1-5 sdp1L-2* (Table I). Indeed, DAG and MAG lipase activities in the mutants are not statistically significantly different from wild type ($P > 0.05$). These data suggest that SDP1 and SDP1L are responsible for more than 90% of the TAG lipase activity associated with the oil body membrane and that other proteins must account for the majority of the DAG and MAG lipase activity measured in this fraction.

SDP1L and *SDP1* Are Expressed in a Range of Tissues

SDP1 and *SDP1L* transcript abundance were measured in various Arabidopsis tissues using real-time PCR (Fig. 4). These data show that *SDP1* and *SDP1L* have a similar pattern of expression in seeds with transcript abundance relatively high during late seed development and in dry seeds, while transcript levels are lower following germination. However, the quantity of *SDP1L* transcript appears to be much lower compared to *SDP1* in seed tissues and also in various vegetative tissues. The only exception is mature pollen where *SDP1L* appears to be much more strongly expressed than *SDP1* (Fig. 4). The patterns of expression revealed by real-time PCR are consistent with published microarray data (Winter et al., 2007). Our data indicate that *SDP1* and *SDP1L* have divergent expression patterns and might therefore play a predominant role in different tissues.

SDP1L Can Partially Complement *sdp1* and Hydrolyses TAG, in Preference to DAG or MAG

SDP1 and SDP1L are approximately 74% identical at the amino acid level (Eastmond, 2006) and lipase assays preformed on oil body membranes from mutant seedling suggest that SDP1L also has TAG lipase

Table I. *Lipase activities associated with oil body membranes purified from 2-day-old wild-type (Col-0), sdp1-5, sdp1-L2, and sdp1-5 sdp1L-2 seedlings*

The seedlings were grown in the light on agar plates containing half-strength Murashige and Skoog salts with 1% (w/v) Suc. [^{3}H]triolein (TAG), [^{3}H]diolein (DAG), and [^{3}H]monolein (MAG) were used as substrates. Values are the mean ± SE of measurements made on four separate preparations and are expressed as a percentage of wild type. The asterisk denotes that the value is significantly different from wild type ($P < 0.05$). The TAG lipase activity in *sdp1-5 sdp1L-2* is also not significantly different from zero ($P > 0.05$).

Genotype	Lipase Activity		
	TAG	DAG	MAG
		% of WT	
WT (Col-0)	100 ± 7.5	100 ± 13.0	100 ± 11.6
sdp1-5	24.3 ± 6.0*	79.5 ± 11.3	94.2 ± 9.2
sdp1L-2	97.0 ± 12.1	89.2 ± 9.5	104.6 ± 11.3
sdp1-5 sdp1L-2	2.6 ± 4.0*	79.9 ± 7.9	97.3 ± 21.5

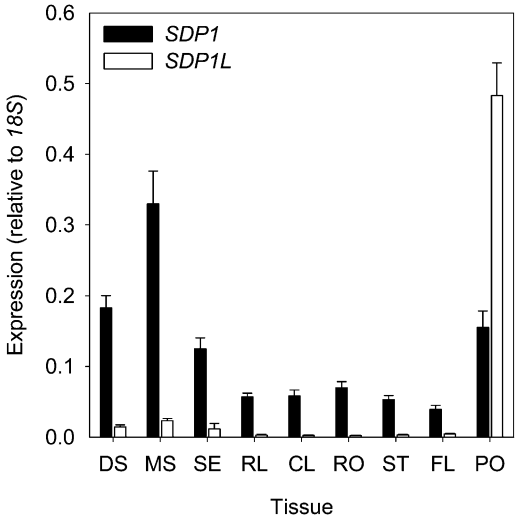

Figure 4. Real-time PCR analysis of *SDP1* and *SDP1L* expression in various Arabidopsis tissues. DS, Developing seed (green cotyledon stage); MS, mature seed; SE, 2-d-old seedling; RL, rosette leaf; CL, cauline leaf; RO, root; ST, stem; FL, flower; PO, mature pollen. Values are the mean ± SE of measurements made on four separate RNA extractions and are normalized relative to *18S* expression.

activity (Table I). To investigate further the extent to which SDP1 and SDP1L are functionally equivalent we created transgenic *sdp1-5* lines containing T-DNA constructs expressing *SDP1* or *SDP1L* under the control of either the constitutive *35S* or approximately 1.5 kb of the *SDP1* promoter. Analysis of seedling hypocotyl growth and 20:1 breakdown in dark-grown 5-d-old seedlings showed that both SDP1 and SDP1L can promote postgerminative growth and TAG breakdown in *sdp1-5* when expressed under the control of either promoter (Fig. 5). However, in the case of both promoters only partial complementation was achieved in lines expressing SDP1L. To measure lipase activity directly in SDP1L and SDP1, hemagglutinin (HA)-tagged proteins were expressed in *sdp1-5*, partially purified by immunoprecipitation (IP), and assayed using [^{3}H]triolein, [^{3}H]diolein, and [^{3}H]monolein as substrates (Table II). Both SDP1L and SDP1 exhibited the highest activity using TAG as a substrate. DAG was also hydrolyzed, but at a comparatively low rate and the rate of MAG hydrolysis was not significantly different from zero ($P > 0.05$). These data suggest that SDP1L has a substrate preference for TAG, over DAG or MAG.

SDP1 and SDP1L Are Important But Not Essential for Seed Germination or Seedling Establishment

Our data suggest that in the *sdp1-5 sdp1L-2* double mutant TAG breakdown during seed germination and early postgerminative growth is reduced to approximately 5% of the rate in wild type (Fig. 3). This rate of breakdown appears to be less than or equivalent to that of the *pxa1-1* and *acx1-2 acx2-1* mutants, which exhibit strongly reduced germination potential and are completely seedling lethal (Zolman et al., 2001; Adham et al., 2005; Baker et al., 2006). To examine the physiological consequences of disruption of *SDP1* and *SDP1L*, seed germination and seedling growth experiments were performed using after-ripened seeds from wild-type and mutant parents that were grown at the same time, under the same conditions. To assess seed germination and seedling growth either cold-stratified (4°C for 4 d) or unstratified seeds were germinated on medium containing one-half Murashige and Skoog salts in the light at 21°C. Under these conditions both the rate and frequency of *sdp1-5 sdp1L-2* seed germination were reduced, relative to wild type. However, within 4 d >90% of *sdp1-5 sdp1L-2* seeds had germinated (Fig. 6).

To assess the impact of SDP1 and SDP1L disruption on seedling growth seeds were cold stratified at 4°C for 4 d and germinated on medium containing one-half Murashige and Skoog salts plus or minus 1% (w/v) Suc in the light or dark at 21°C. After 5 d *sdp1-5*

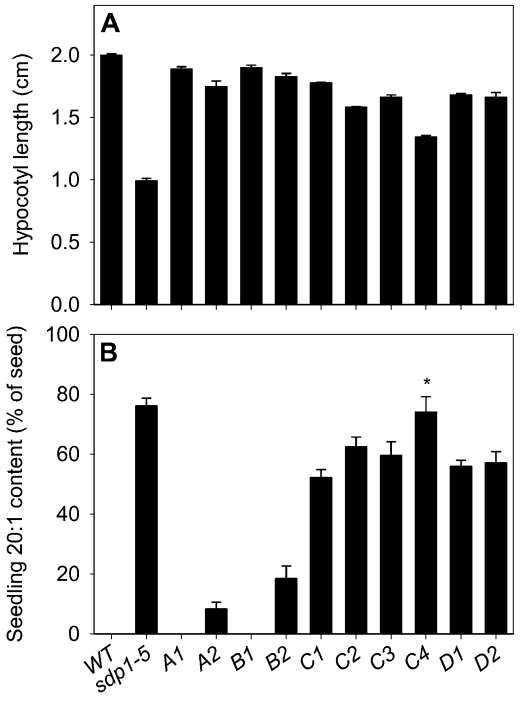

Figure 5. Complementation of *sdp1-5* by expressing SDP1 or SDP1L under the control of either the *SDP1* or the 35S promoter. A1 and 2 = *SDP1p*::*SDP1*, B1 and 2 = *35S*::*SDP1*, C1, 2, 3, and 4 = *SDP1p*::*SDP1L*, and D1 and 2 = *35S*::*SDP1L*. A, Hypocotyl length of 5-d-old seedlings grown in the dark on agar plates containing half-strength Murashige and Skoog salts. Values are the mean ± SE of measurements made on four separate batches of 10 seedlings. B, Eicosenoic acid (20:1) content of 5-d-old seedlings grown in the dark on agar plates containing half-strength Murashige and Skoog salts, expressed as a percentage of the amount present in the same number of seeds prior to germination. Values are the mean ± SE of measurements made on four separate batches of 20 seeds or seedlings plus seed coats. The asterisk denotes that the value does not significantly differ from *sdp1-5* ($P > 0.05$). Where bars are absent no 20:1 was detected in 5-d-old seedlings.

Table II. *Lipase activities associated with SDP1 and SDP1L*

HA-tagged SDP1 and SDP1L were expressed in *sdp1-5* under the control of the *SDP1* promoter and lipase assays were performed on IPs from seedling extracts using [^{3}H]triolein (TAG), [^{3}H]diolein (DAG), and [^{3}H]monolein (MAG) as substrates. Values are the mean ± SE of measurements made on IPs from four extracts containing the same number of seedlings. IP from *sdp1-5* was used as a negative control. The asterisk denotes that the value is significantly different from zero ($P < 0.05$).

Genotype	Lipase Activity		
	TAG	DAG	MAG
		nmol h^{-1}	
sdp1-5 (-ve control)	2.7 ± 4.1	4.1 ± 6.0	2.3 ± 4.9
sdp1-5 SDP1p::SDP1-HA	1,247.5 ± 32.1*	139.7 ± 9.0*	2.0 ± 3.1
sdp1-5 SDP1p::SDP1L-HA	339.5 ± 8.7*	56.5 ± 2.8*	3.1 ± 4.2

sdp1L-2 double-mutant seedlings showed a retarded growth phenotype both in the dark (measured as hypocotyl length) and in the light (measured as root growth) that was more pronounced than that of *sdp1-5* and could be rescued by Suc (Fig. 7, A and B). The *sdp1L-2* single mutant (Fig. 7) and *atglL* and *cgi58L* (data not shown) did not exhibit any obvious seedling growth phenotype under these conditions. Although *sdp1-5 sdp1L-2* seedling growth was severely retarded after 5 d on one-half Murashige and Skoog medium in the light, by 14 d approximately 20% of the seedlings were able to establish themselves, as defined by the expansion of true leaves and development of a root system (Fig. 7C). When *sdp1-5 sdp1L-2* seeds were sown on the surface of soil in the glasshouse a substantial proportion of the seedlings were also able to establish (data not shown).

DISCUSSION

In this study we show that SDP1L is responsible for virtually all the remaining TAG hydrolysis that is observed following seed germination in the *sdp1* mutant. Some residual breakdown was observed in *sdp1 sdp1L* seedlings, particularly when they were grown in the light. This rate of breakdown still represented <5% of the maximum rate attained in wild type. TAG lipase activity was also reduced by more than 90% in oil body membranes purified from *sdp1 sdp1L* seedlings. However, the existence of DAG and MAG lipase activity associated with these membranes strongly suggests that additional lipases, whose molecular identities are unknown, are most likely to work in concert with SDP1 and SDP1L to completely hydrolyze TAG to free fatty acid and glycerol. Such activities have previously been detected in oil body membranes from several oilseed species including oilseed rape (*Brassica napus*; Lin and Huang, 1983), which is a close relative of Arabidopsis. In mammalian adipocytes TAG is completely hydrolyzed to free fatty acid and glycerol by the sequential action of ATGL, hormone-sensitive lipase, and MAG lipase (Zechner et al., 2009). Genetic evidence also suggests that complete TAG hydrolysis occurs in Arabidopsis since the *glycerol insensitive1* mutant, which is deficient in glycerol catabolism owing to a defect in glycerol kinase, accumulates a quantity of free glycerol in its seedlings that is roughly equivalent to the amount originally stored in the form of TAG prior to germination (Eastmond, 2004). There are more than 200 genes in Arabidopsis that are annotated as either lipases or acyl-hydrolases and among these 13 have recently been listed as possible TAG or DAG lipases and 16 as possible MAG lipases (Li-Beisson et al., 2010). Further work will be required to

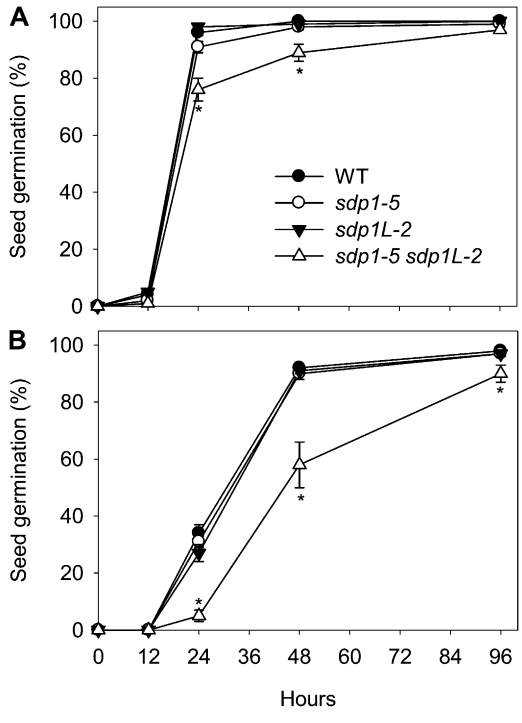

Figure 6. Seed germination in *sdp1-5*, *sdp1-L2*, and *sdp1-5 sdp1-L2*. Seeds of each genotype were placed on agar plates containing half-strength Murashige and Skoog salts and transferred to a growth room with (A) or without (B) a prior stratification treatment of 4 d at 4°C. The percentage of seeds that had germinated (radicles emerged) was scored over the course of 4 d. Values are the mean ± SE of measurements made on four batches of approximately 100 seedlings. The asterisk denotes that the value significantly differs from wild type ($P < 0.05$).

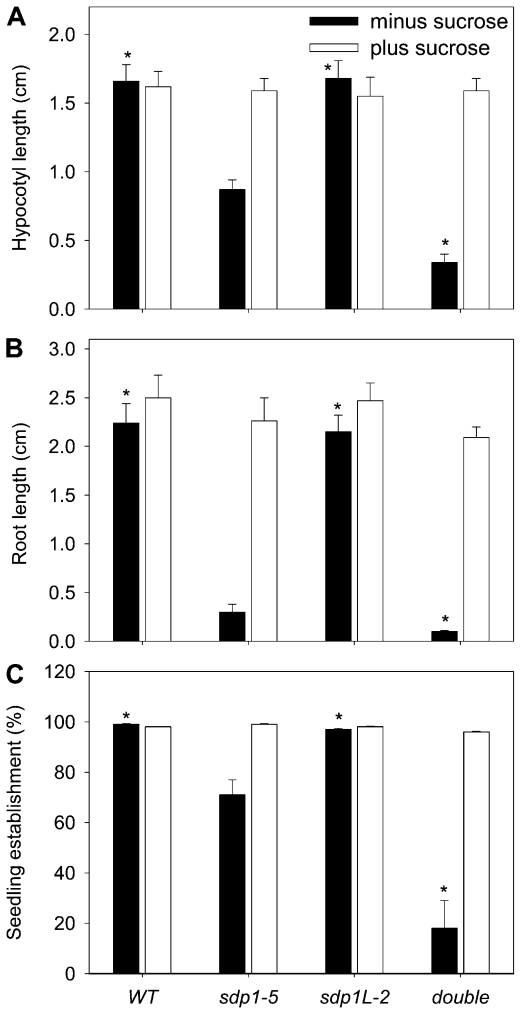

Figure 7. Postgerminative growth and seedling establishment in *sdp1-5*, *sdp1-L2*, and *sdp1-5 sdp1-L2*. A, Hypocotyl length of 5-d-old seedlings grown in the dark on agar plates containing half-strength Murashige and Skoog salts either with or without 1% (w/v) Suc. Values are the mean ± SE of measurements made on four separate batches of 20 seedlings. B, Root length of 7-d-old seedlings grown in a 16-h light (approximately 150 μmol m^{-2} s^{-1})/8-h dark regime on agar plates containing half-strength Murashige and Skoog salts either with or without 1% (w/v) Suc. Values are the mean ± SE of measurements made on three separate batches of 10 seedlings. C, Percentage seedling establishment (defined as development of green expanded true leaves) after 14 d growth in a 16-h light (approximately 150 μmol m^{-2} s^{-1})/8-h dark regime on agar plates containing half-strength Murashige and Skoog salts either with or without 1% (w/v) Suc. Values are the mean ± SE of measurements made on four batches of approximately 100 seedlings. The asterisk denotes that the value significantly differs from *sdp1-5* minus Suc ($P < 0.05$).

determine which of these genes play a physiological role in DAG and MAG breakdown following Arabidopsis seed germination. The residual TAG hydrolysis that is also observed in *sdp1 sdp1L* seedlings might be caused by substrate promiscuity among DAG and MAG lipases, by other lipases/esterases that may not normally play a quantitatively significant role in TAG breakdown, or conceivably by enzymes involved in TAG synthesis such as acyl-CoA:DAG acyltransferase or phospholipid:DAG acyltransferase, which might catalyze a reverse reaction (Feussner et al., 2001). It is important to note that less than 20% of the TAG lipase activity that is measurable in Arabidopsis seedlings is associated with the oil body membrane fraction and the identity and role of the proteins responsible for the remaining activity remain to be determined (El-Kouhen et al., 2005; Eastmond, 2006). Finally, a fractional loss of TAG following seed germination might also be accounted for through mechanical damage to the tissues during harvesting.

A comparison of *sdp1*, *sdp1L*, and *sdp1 sdp1L* demonstrates that SDP1 makes the major contribution to TAG breakdown following seed germination. This is consistent with the observation that *SDP1* is much more strongly expressed in seeds than *SDP1L*. Nevertheless, SDP1L can partially complement the *sdp1* mutant, when expressed under the control of either the 35S or *SDP1* promoter. Therefore, SDP1L function must be broadly equivalent to that of SDP1 (Eastmond, 2006). SDP1L might be unable to fully complement TAG breakdown in *sdp1* because of a lower specific activity, differences in substrate specificity, or differences in posttranscriptional/translational regulation. Analysis of the partially complemented lines also shows that, under the laboratory conditions described here, TAG breakdown in Arabidopsis can be substantially reduced without a major impact on hypocotyl growth in the dark. Indeed retention of approximately 50% of TAG in 5-d-old dark-grown seedlings results in only an approximately 10% reduction in hypocotyl length (Fig. 5). These findings have implications for the design of forward and reverse genetic screens intended to identify mutants in TAG breakdown (Eastmond et al., 2000; Eastmond, 2006) since mutants with significant defects in TAG breakdown are likely to exist that do not exhibit an obvious impairment in postgerminative growth.

It appears that SDP1L functions redundantly with regard to the phenotypes investigated in this study. Interestingly in yeast the three PTLs (TGL3, TGL4, and TGL5) do not function redundantly. Disruption of either TGL3 or TGL4 impairs TAG breakdown (Athenstaedt and Daum, 2003, 2005; Kurat et al., 2006) and TGL3 has been reported to hydrolyze both TAG and DAG in vitro, whereas TGL4 is only active on TAG (Kurat et al., 2006). Phylogenetic analysis suggests that both SDP1 and SDP1L are more closely related to TGL4 than to TGL3 (Eastmond, 2006), which would be consistent with our in vitro data, which suggest that both SDP1 and SDP1L are more active on TAG than on DAG or MAG (Eastmond, 2006; Table II). The observation that oil body membranes from *sdp1 sdp1L* seedlings lack TAG lipase activity but contain significant levels of DAG and MAG lipase activity also broadly supports this hypothesis. TGL3, TGL4, and TGL5 have also been reported to exhibit various other

enzyme activities in recent studies (Rajakumari and Daum, 2010a, 2010b). Therefore it remains possible that SDP1 and SDP1L might have as-yet-undiscovered biochemical functions in addition to TAG hydrolysis.

SDP1L is most strongly expressed in pollen. Oil bodies are known to be highly abundant in maturing pollen and also in the tapetal cells of the anther (Kim et al., 2002). The oil bodies in pollen rapidly disappear following germination (Rodríguez-García et al., 2003) and recent studies suggest that TAG synthesis is essential for pollen development in Arabidopsis (Zhang et al., 2009), while fatty acid catabolism is also important for normal pollen germination and pollen tube growth (Footitt et al., 2007). It is possible that the SDP1L isoform might therefore play a predominant role in pollen, perhaps by providing carbon skeletons to support stigma penetration and pollen tube growth. The *sdp1*, *sdp1L*, and *sdp1 sdp1L* mutants are all fertile and no increase in the frequency of unfertilized ovules was observed in siliques from self-fertilized plants (data not shown). However, it remains possible that the mutant pollen could exhibit some defects in comparison to wild type (Footitt et al., 2007). Further work will be required to investigate the role of SDP1 and SDP1L in pollen.

Although *sdp1 sdp1L* seeds are almost completely blocked in TAG breakdown there is relatively little detrimental effect on seed germination under the conditions used in this study. This is in contrast to several mutants that are defective in peroxisomal fatty acid import or β-oxidation, which have been reported to exhibit strongly reduced germination potential (Footitt et al., 2002; Baker et al., 2006). Indeed we show that *sdp1 sdp1L*, *pxa1*, and *acx1 acx2* seeds have a very similar capacity for fatty acid breakdown following germination. It has previously been suggested that the poor germination phenotype of *pxa1* and *acx1 acx2* may not be due to a shortage of carbon skeletons to support growth (Baker et al., 2006) and our analysis of *sdp1 sdp1L* supports this argument. Baker et al. (2006) have hypothesized that products or precursors of peroxisomal β-oxidation that are not derived from TAG hydrolysis might be required to promote or repress seed germination and seedling growth. In support of this proposal Dave et al. (2011) have recently shown that the jasmonic acid precursor 12-oxophytodienoic acid accumulates in *pxa1* and *acx1 acx2* seeds and can also inhibit germination when applied exogenously to wild-type seeds.

Our data show that Arabidopsis seedling establishment is also possible with very little TAG breakdown (a peak rate that is approximately 5% of wild type). However, even under optimal laboratory conditions growth is severely retarded and the frequency of seedling survival is substantially reduced. Arabidopsis seeds contain substantial quantities of protein and some soluble carbohydrates (Baud et al., 2002). It is likely that *sdp1 sdp1L* seedlings utilize these reserves to establish photosynthetic competence in some (or all) of their cotyledon cells and, once net carbon fixation commences, these cells are able to export carbon skeletons to the rest of the seedling tissues and ultimately allow growth to resume. It should be noted that burial of *sdp1 sdp1L* seeds to a depth of approximately 5 mm in soil completely prevents seedling establishment (data not shown). Unlike Arabidopsis, whose seed germination is light dependent, many oilseed species germinate in darkness beneath the soil. The severity of the *sdp1 sdp1L* seedling establishment phenotype is quite similar to that of the glyoxylate cycle mutant *icl*, which lacks isocitrate lyase and is therefore also unable to synthesize carbohydrates from fatty acids (Eastmond et al., 2000). Cornah et al. (2004) have shown that sugar levels are depressed in *icl* seedlings and that many genes classically associated with carbohydrate starvation (Thimm et al., 2004) are up-regulated, including those encoding enzymes involved in carbohydrate, protein, and amino acid degradation. The capacity of Arabidopsis to use protein as a source of carbon skeletons may not be surprising since protein is the major seed storage compound in some plant species. For example, in *Medicago truncatula* protein accounts for up to 45% of the seed weight, while TAG accounts for only 12%, and starch $<1\%$ (Gallardo et al., 2003).

In contrast to SDP1 and SDP1L, our mutant analysis suggests that ATGLL or CGI58L are unlikely to play a quantitatively important role in TAG breakdown following seed germination. The physiological role of ATGLL is currently unknown. However, CGI58L has been the subject of two recent studies (Ghosh et al., 2009; James et al., 2010). James et al. (2010) recently showed that disruption of CGI58L does not impair seedling establishment, but that it does cause TAG accumulation in leaves and therefore the protein does play a role in TAG hydrolysis. In mammals, CGI-58 activates ATGL through protein-protein interaction (Lass et al., 2006). Yeast also contains a homolog of CGI-58 called ICT1 (Ghosh et al., 2008b). However, currently it is not known whether ICT1 can activate TGL3, 4, or 5. Since SDP1 and SDP1L are more closely related to TGL3, 4, and 5 than to ATGL (Eastmond, 2006) it is possible that CGI58L might not be an activator of these lipases, although further work will be necessary to resolve this question. Mammalian CGI-58 is an α/β-hydrolase fold-containing protein but it is not an active hydrolase since the catalytic Ser in the GXSXG motif is substituted for another amino acid. In contrast, the catalytic Ser is conserved in ICT1 and in Arabidopsis CGI58L and these proteins have both been reported to exhibit lipase activity (Ghosh et al., 2008b, 2009). On this basis CGI58L might hydrolyze TAG directly in leaves (James et al., 2010). Finally, CGI-58, ICT1, and CGI58L also possess an acyltransferase domain, composed of a His and an Asp, separated by four less-conserved residues (HXXXXD; Ghosh et al., 2008a, 2008b, 2009). This domain has been reported to confer a lysophosphatidic acid acyltransferase activity and consequently the proteins have also been implicated in phospholipid biosynthesis (Ghosh et al., 2008a, 2008b, 2009; Montero-Moran et al., 2010).

In light of these findings, it will be interesting to investigate whether SDP1, SDP1L, or ATGLL play any role in lipid homeostasis in vegetative tissues.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Wild-type Arabidopsis (*Arabidopsis thaliana*; ecotype Col-0 and Wassilewskija) and the T-DNA mutants *sdp1L-2* (N873426), *atglL* (N655182), and *cgi58L* (N679146) were obtained from the European Arabidopsis Stock Centre. The *sdp1L-1* mutant (FLAG_523C11) was obtained from the Versailles Genetics and Plant Breeding Laboratory *Arabidopsis thaliana* Resource Centre. The *sdp1-4* and *sdp1-5* mutants are described in Eastmond (2006). For seedling growth experiments the seeds were surface sterilized, applied to agar plates containing one-half strength Murashige and Skoog salts (Sigma-Aldrich) plus or minus 1% (w/v) Suc, and imbibed in the dark for 4 d at 4°C. The plates were then transferred to a growth chamber set to 21°C (16-h light/8-h dark; photosynthetic photon flux density = 150 μmol m^{-2} s^{-1}). For etiolated seedlings the plates were kept in darkness in the growth chamber after 1 h exposure to light. For seed germination experiments germination was scored as radicle emergence from the seed coat.

Transcript Analysis

DNAse-treated total RNA was isolated from various Arabidopsis tissues using either the RNeasy kit from Qiagen Ltd., or the method of Wu et al. (2002). The synthesis of single-stranded cDNA was carried out using SuperScriptTM II RNase H- reverse transcriptase from Invitrogen Ltd. *SDP1*, *SDP1-L*, *ATGL-L*, *CGI58-L*, and *ACTIN2* transcripts were detected by PCR using the primers pairs SDP1F (5′-GAATCAATAATGGATATAAGTAATGAGGCTAGTGT-3′) and SDP1R (5′-AGCATCTATAACACTACCAGACACCGGTTC-3′), SDP1LF (5′-ATGGATATAAGCAACCGAAGCAGGC-3′) and SDP1LR (5′-CTCATGGTCATGGATTAGATTCG-3′), ATGLLF (5′-GAGAGGTTACTGCCCGATGATATTCACA-3′) and ATGLLR (5′-TATCGTCATAGACCAATCCCTCAACTGG-3′), CGI58LF (5′-ATGAACTTGAGCCGTTTTGCTTCGAG-3′) and CGI58LR (5′-CCAAGTTGATCAATAGCGATCACTCTAAATCG-3′), and ACT2F (5′-GTTGGGATGAACCAGAAGGA-3′) and ACT2R (5′-CTTACAATTTCCCGCTCTGC-3′). Quantitative real-time PCR was performed with an ABI Prism 7000 sequence detection system (Applied Biosystems) using SYBR green PCR master mix as described by Penfield et al. (2004). The primer pairs used for real-time PCR were QSDP1F (5′-AAATGGCTTACCGGAGGAAGTT-3′) plus QSDP1R (5′-TGAGCCCATTCCTCATAAGTCA-3′), QSDP1LF (5′-CCTTAAACGCAGCGCAGAA-3′) plus QSDP1LR (5′-GATGTTTAGATGGTGGAGAGGAAGA-3′), and 18SF plus 18SR (Penfield et al., 2004).

Determination of Lipids and Lipase Activity

The fatty acid composition of seed and seedling total lipids and TAG were measured by gas chromatographic analysis of fatty acid methyl ester using the methods described by Browse et al. (1986) and Eastmond (2006), respectively. Oil body membranes were purified from 2-d-old seedlings and lipase activities were measured using an emulsion of [9,10-^{3}H]triolein, [9,10-^{3}H]diolein, or [9,10-^{3}H]monolein, as described previously (Eastmond, 2006). For IP experiments 2-week-old seedlings were ground in 50 mM Bis-Tris propane/HCl (pH 8), 2 mM dithiothreitol, and 2 mM $CaCl_2$ plus plant protease inhibitor cocktail (Sigma-Aldrich). Extracts were spun for 5 min at 12,000*g* and the supernatants were used directly in IP reactions. One-milliliter aliquots of extract were precleared by the addition of 20 μL of protein A-agarose beads (Sigma-Aldrich), incubation for 30 min, and spun at 5,000*g* for 5 min. Twenty microliters of anti-HA (3F10) agarose beads (Roche) were added to the precleared extract, and the mixture was incubated with constant mixing for 1 h, and the beads were pelleted by spinning at 5,000*g* for 30 s at 4°C. The beads were washed four times in buffer, resuspended, and lipase activities were measured as described above. [9,10-^{3}H]diolein and [9,10-^{3}H]monolein were prepared from [9,10-^{3}H]triolein (PerkinElmer) following partial hydrolysis with pancreatic lipase (Sigma-Aldrich) and separation by thin-layer chromatography (Xu et al., 2005).

Creation of DNA Constructs and Arabidopsis Transformation

SDP1, *SDP1L*, and approximately 1.5 kb of the *SDP1* promoter were amplified by PCR from Arabidopsis cDNA using primer pairs 5′-CACCATGGATATAAGTAATGAGGCTAGT-3′ and 5′-AGCATCTATAACACTACCAGACACCGGTT-3′, 5′-CACCATGGATATAAGCAACGAAGCAGGCGTTG-3′ and 5′-CTCATGGTCATGGTCATGGATTAGATTCGCCTGAGC-3′ and 5′-CCATGGTTCGAGTTTTATTTTCGTTACTTCCAATCA-3′ and 5′-CCATGGTATTGATTCGAAGATGAATTTGGGTGTGT-3′, respectively. *SDP1* and *SDP1L* were cloned into the pENTR/D-TOPO vector, and the *SDP1* promoter was cloned into the TA vector pCR2.1, according to the manufacturer's instructions (Invitrogen Ltd.). The *SDP1* promoter was then excised from pCR2.1 using *Nco*I and ligated into the two pENTR/D-TOPO vectors at the *Nco*I site using T4 DNA ligase. The resulting promoter gene fusions were then transferred to the binary vector pGWB13 using the Gateway LR clonase enzyme mix, according to the manufacturer's instructions (Invitrogen Ltd.). *SDP1* and *SDP1L* in the pENTR/D-TOPO vector were also transferred to binary vectors containing a 35S promoter (CTAPi and pB2GW7, respectively). The constructs were transformed into *Agrobacterium tumefaciens* strain GV3101 by heat shock and into *sdp1-5* by the floral-dip method (Clough and Bent, 1998). Transformants containing T-DNA insertions were selected via antibiotic or herbicide resistance and hypocotyl length (Eastmond, 2006).

Statistical Analyses

Fatty acid content, TAG content, hypocotyl length, and root length were compared among genotypes using paired *t* tests assuming unequal variance. One-sample *t* tests were performed to compare values to zero and in some cases post-hoc statistical power calculations were made to determine minimum detectable difference.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers NM_120486 (SDP1), NM_202720 (SDP1L), NM_202228 (ATGLL), and NM_202876 (CGI58L).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Hypocotyl length and eicosenoic acid content of *sdp1-4*, *sdp1L-1*, and *sdp1-4 sdp1L-1* seedlings.

Supplemental Figure S2. Time course of eicosenoic acid breakdown after seed germination in wild type, *sdp1L-2*, *atglL*, and *cgi58L*.

Received June 15, 2011; accepted August 4, 2011; published August 8, 2011.

LITERATURE CITED

Adham AR, Zolman BK, Millius A, Bartel B (2005) Mutations in Arabidopsis acyl-CoA oxidase genes reveal distinct and overlapping roles in beta-oxidation. Plant J **41:** 859–874

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana.* Science **301:** 653–657

Athenstaedt K, Daum G (2003) YMR313c/TGL3 encodes a novel triacylglycerol lipase located in lipid particles of *Saccharomyces cerevisiae.* J Biol Chem **278:** 23317–23323

Athenstaedt K, Daum G (2005) Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* are localized to lipid particles. J Biol Chem **280:** 37301–37309

Baker A, Graham IA, Holdsworth M, Smith SM, Theodoulou FL (2006) Chewing the fat: β-oxidation in signalling and development. Trends Plant Sci **11:** 124–132

Baud S, Boutin J-P, Miquel M, Lepiniec L, Rochat C (2002) An integrated overview of seed development in *Arabidopsis thaliana* ecotype Ws. Plant Physiol Biochem **40:** 151–160

Bewley JD, Black M (1994) Seeds. Physiology of Development and Germination, Ed 2. Plenum Press, New York

Browse J, McCourt PJ, Somerville CR (1986) Fatty acid composition of leaf

lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Anal Biochem **152:** 141–145

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735–743

Cornah JE, Germain V, Ward JL, Beale MH, Smith SM (2004) Lipid utilization, gluconeogenesis, and seedling growth in Arabidopsis mutants lacking the glyoxylate cycle enzyme malate synthase. J Biol Chem **279:** 42916–42923

Dave AM, Hernández ML, He Z, Andriotis VME, Vaistij FE, Larson TR, Graham IA (2011) 12-Oxo-phytodienoic acid accumulation during seed development represses seed germination in *Arabidopsis*. Plant Cell **23:** 583–599

Eastmond PJ (2004) Glycerol-insensitive Arabidopsis mutants: *gli1* seedlings lack glycerol kinase, accumulate glycerol and are more resistant to abiotic stress. Plant J **37:** 617–625

Eastmond PJ (2006) *SUGAR-DEPENDENT1* encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating *Arabidopsis* seeds. Plant Cell **18:** 665–675

Eastmond PJ, Germain V, Lange PR, Bryce JH, Smith SM, Graham IA (2000) Postgerminative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle. Proc Natl Acad Sci USA **97:** 5669–5674

El-Kouhen K, Blangy S, Ortiz E, Gardies AM, Ferté N, Arondel V (2005) Identification and characterization of a triacylglycerol lipase in Arabidopsis homologous to mammalian acid lipases. FEBS Lett **579:** 6067–6073

Feussner I, Kühn H, Wasternack C (2001) Lipoxygenase-dependent degradation of storage lipids. Trends Plant Sci **6:** 268–273

Footitt S, Dietrich D, Fait A, Fernie AR, Holdsworth MJ, Baker A, Theodoulou FL (2007) The COMATOSE ATP-binding cassette transporter is required for full fertility in Arabidopsis. Plant Physiol **144:** 1467–1480

Footitt S, Slocombe SP, Larner V, Kurup S, Wu Y, Larson T, Graham I, Baker A, Holdsworth M (2002) Control of germination and lipid mobilization by COMATOSE, the Arabidopsis homologue of human ALDP. EMBO J **21:** 2912–2922

Gallardo K, Le Signor C, Vandekerckhove J, Thompson RD, Burstin J (2003) Proteomics of *Medicago truncatula* seed development establishes the time frame of diverse metabolic processes related to reserve accumulation. Plant Physiol **133:** 664–682

Ghosh AK, Chauhan N, Rajakumari S, Daum G, Rajasekharan R (2009) At4g24160, a soluble acyl-coenzyme A-dependent lysophosphatidic acid acyltransferase. Plant Physiol **151:** 869–881

Ghosh AK, Ramakrishnan G, Chandramohan C, Rajasekharan R (2008a) CGI-58, the causative gene for Chanarin-Dorfman syndrome, mediates acylation of lysophosphatidic acid. J Biol Chem **283:** 24525–24533

Ghosh AK, Ramakrishnan G, Rajasekharan R (2008b) YLR099C (ICT1) encodes a soluble Acyl-CoA-dependent lysophosphatidic acid acyltransferase responsible for enhanced phospholipid synthesis on organic solvent stress in *Saccharomyces cerevisiae*. J Biol Chem **283:** 9768–9775

Graham IA (2008) Seed storage oil mobilization. Annu Rev Plant Biol **59:** 115–142

Grönke S, Mildner A, Fellert S, Tennagels N, Petry S, Müller G, Jäckle H, Kühnlein RP (2005) Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab **1:** 323–330

James CN, Horn PJ, Case CR, Gidda SK, Zhang D, Mullen RT, Dyer JM, Anderson RG, Chapman KD (2010) Disruption of the Arabidopsis CGI-58 homologue produces Chanarin-Dorfman-like lipid droplet accumulation in plants. Proc Natl Acad Sci USA **107:** 17833–17838

Kim HU, Hsieh K, Ratnayake C, Huang AH (2002) A novel group of oleosins is present inside the pollen of Arabidopsis. J Biol Chem **277:** 22677–22684

Kurat CF, Natter K, Petschnigg J, Wolinski H, Scheuringer K, Scholz H, Zimmermann R, Leber R, Zechner R, Kohlwein SD (2006) Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast. J Biol Chem **281:** 491–500

Lass A, Zimmermann R, Haemmerle G, Riederer M, Schoiswohl G, Schweiger M, Kienesberger P, Strauss JG, Gorkiewicz G, Zechner R (2006) Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman syndrome. Cell Metab **3:** 309–319

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

Li-Beisson Y, Shorrosh B, Beisson F, Andersson M, Arondel V, Bates PD, Baud S, Bird D, DeBono A, Durrett TP, et al (2010) Acyl-lipid metabolism. *In* R Last, ed, The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, pp 1–65

Lin YH, Huang AHC (1983) Lipase in lipid bodies of cotyledons of rape and mustard seedlings. Arch Biochem Biophys **225:** 360–369

Montero-Moran G, Caviglia JM, McMahon D, Rothenberg A, Subramanian V, Xu Z, Lara-Gonzalez S, Storch J, Carman GM, Brasaemle DL (2010) CGI-58/ABHD5 is a coenzyme A-dependent lysophosphatidic acid acyltransferase. J Lipid Res **51:** 709–719

Penfield S, Rylott EL, Gilday AD, Graham S, Larson TR, Graham IA (2004) Reserve mobilization in the *Arabidopsis* endosperm fuels hypocotyl elongation in the dark, is independent of abscisic acid, and requires PHOSPHOENOLPYRUVATE CARBOXYKINASE1. Plant Cell **16:** 2705–2718

Quettier AL, Eastmond PJ (2009) Storage oil hydrolysis during early seedling growth. Plant Physiol Biochem **47:** 485–490

Rajakumari S, Daum G (2010a) Janus-faced enzymes yeast Tgl3p and Tgl5p catalyze lipase and acyltransferase reactions. Mol Biol Cell **21:** 501–510

Rajakumari S, Daum G (2010b) Multiple functions as lipase, steryl ester hydrolase, phospholipase, and acyltransferase of Tgl4p from the yeast *Saccharomyces cerevisiae*. J Biol Chem **285:** 15769–15776

Rodríguez-García MI, M'rani-Alaoui M, Fernández MC (2003) Behavior of storage lipids during development and germination of olive (*Olea europaea* L.) pollen. Protoplasma **221:** 237–244

Roudier F, Gissot L, Beaudoin F, Haslam R, Michaelson L, Marion J, Molino D, Lima A, Bach L, Morin H, et al (2010) Very-long-chain fatty acids are involved in polar auxin transport and developmental patterning in *Arabidopsis*. Plant Cell **22:** 364–375

Smirnova E, Goldberg EB, Makarova KS, Lin L, Brown WJ, Jackson CL (2006) ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells. EMBO Rep **7:** 106–113

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J **37:** 914–939

Winter D, Vinegar B, Hardeep N, Ammar R, Wilson GV, Provart NJ (2007) An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS ONE **2:** e718

Wu Y, Llewellyn DJ, Dennis ES (2002) A quick and easy method for isolating good quality RNA from cotton (*Gossypium hirsutum* L.) tissues. Plant Mol Biol Rep **20:** 213–218

Xu C, Fan J, Froehlich JE, Awai K, Benning C (2005) Mutation of the TGD1 chloroplast envelope protein affects phosphatidate metabolism in *Arabidopsis*. Plant Cell **17:** 3094–3110

Zhang M, Fan J, Taylor DC, Ohlrogge JB (2009) DGAT1 and PDAT1 acyltransferases have overlapping functions in *Arabidopsis* triacylglycerol biosynthesis and are essential for normal pollen and seed development. Plant Cell **21:** 3885–3901

Zechner R, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A (2009) Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. J Lipid Res **50:** 3–21

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, et al (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science **306:** 1383–1386

Zolman BK, Silva ID, Bartel B (2001) The Arabidopsis *pxa1* mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid beta-oxidation. Plant Physiol **127:** 1266–1278