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-DÉPENDENT1 (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...
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; 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 period coincides with seed germination and the onset of TAG hydrolysis during postgerminative seedling growth, and establishment.

Figure 1. Isolation of T-DNA mutants in SDP1L, ATGLL, and CGI58L. A, Representation of the SDP1L, 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 sdp1L-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.

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, sdp1L-4 and sdp1L-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 Wasselewskaja allele sdp1L-1 behaved the same as sdp1L-2 as a single mutant and when combined with sdp1L-4 (Supplemental Fig. S1).
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).

For comparison fatty acid breakdown was also measured in the pxa1-2 and acx1-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 pxa1-2 and acx1-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-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).

**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).

**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,
sdp1L-2, and the double mutant using [3H]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 [3H]diolein and [3H]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 performed on oil body membranes from mutant seedling suggest that SDP1L also has TAG lipase

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**Table I. Lipase activities associated with oil body membranes purified from 2-day-old wild-type (Col-0), sdp1-5, sdp1L-2, 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. [3H]triolein (TAG), [3H]diolein (DAG), and [3H]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*     | 89.5 ± 9.5      | 104.6 ± 11.3    |
| sdp1L-2           | 97.0 ± 12.1     | 97.9 ± 7.9      | 104.6 ± 11.3    |
| sdp1-5 sdp1L-2    | 2.6 ± 4.0*      | 97.3 ± 21.5     |                 |

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[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.]
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 [3H]triolein, [3H]diolein, and [3H]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 4](image-url) 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.

![Figure 5](image-url) 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.
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 cgis8l (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

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

| Genotype                  | Lipase Activity | Lipase Activity |
|--------------------------|-----------------|-----------------|
|                           | TAG             | DAG             | MAG             |
|                           | mmol h$^{-1}$   | mmol h$^{-1}$   | mmol 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       |

Figure 6. Seed germination in sdp1-5, sdp1L-2, and sdp1-5 sdp1L-2. 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 ± s of measurements made on four batches of approximately 100 seedlings. The asterisk denotes that the value significantly differs from wild type ($P < 0.05$).
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 

\( sdp1 \) \( sdp1\) 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 \( sdp1 \), \( sdp1\), and \( sdp1 \) \( sdp1\) 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 \( sdp1 \) \( sdp1\) 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

Figure 7. Postgerminative growth and seedling establishment in \( sdp1-5 \), \( sdp1-2 \), and \( sdp1-5 \) \( sdp1-2\). 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 \( \mu \)mol m\textsuperscript{-2} s\textsuperscript{-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 \( \mu \)mol m\textsuperscript{-2} s\textsuperscript{-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 (\( P < 0.05 \)).
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 (Rodriguez-Garcia 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 GNSXG 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 ATGL2 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 sdpl-2 (N873426), atglL (N655182), and cgi58L (N679146) were obtained from the European Arabidopsis Stock Centre. The sdpl-1 mutant (FLAG_52C11) was obtained from the Versailles Genetics and Plant Breeding Laboratory Arabidopsis thaliana Resource Centre. The sdpl-4 and sdpl-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 light/18°C 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 3 h exposure to light. For seed germination experiments germination was scored as radicle emergence from the seed coat.

Transcript Analysis

DNA-seq-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 RNSase H- reverse transcriptase from Invitrogen Ltd. SDP1, SDP1L, ATGL2, CGI58L, and ACTIN2 transcripts were detected by PCR using the RNeasy kit from Qiagen Ltd., or the method of Wu et al. (2003) for etiolated seedlings the plates were kept in darkness in the growth chamber after 3 h exposure to light. For seed germination experiments germination was scored as radicle emergence from the seed coat.

Supplemental Data

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

Supplemental Figure S1. Hypocotyl length and eicosonic acid content of sdpl-4, sdpl-1, and sdpl-4 sdpl1-1 seedlings.

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

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

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