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Abstract: Storage oil breakdown plays an important role in the life cycle of many plants by providing the carbon skeletons that support seedling growth immediately following germination. This metabolic process is initiated by lipases (EC: 3.1.1.3), which catalyse the hydrolysis of triacylglycerols (TAGs) to release free fatty acids and glycerol. A number of lipases have been purified to near homogeneity from seed tissues and analysed for their in vitro activities. Furthermore, several genes encoding lipases have been cloned and characterized from plants. However, only recently has data been presented to establish the molecular identity of a lipase that has been shown to be required for TAG breakdown in seeds. In this review we briefly outline the processes of TAG synthesis and breakdown. We then discuss some of the biochemical literature on seed lipases and describe the cloning and characterization of a lipase called SUGAR-DEPENDENT1, which is required for TAG breakdown in Arabidopsis thaliana seeds.

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Storage oil hydrolysis during early seedling growth

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Abstract

Storage oil breakdown plays an important role in the life cycle of many plants by providing the carbon skeletons that support seedling growth immediately following germination. This metabolic process is initiated by lipases (EC: 3.1.1.3), which catalyse the hydrolysis of triacylglycerols (TAGs) to release free fatty acids and glycerol. A number of lipases have been purified to near homogeneity from seed tissues and analysed for their in vitro activities. Furthermore, several genes encoding lipases have been cloned and characterized from plants. However, only recently has data been presented to establish the molecular identity of a lipase that has been shown to be required for TAG breakdown in seeds. In this review we briefly outline the processes of TAG synthesis and breakdown. We then discuss some of the biochemical literature on seed lipases and describe the cloning and characterization of a lipase called SUGAR-DEPENDENT1, which is required for TAG breakdown in *Arabidopsis thaliana* seeds.

Keywords: ATGL, adipose triglyceride lipase; PLD, patatin-like domain; PTL, patatin-like TAG lipase; SDP, sugar-dependent; TAG, triacylglycerol, TGL, triacylglycerol lipase.

1. Introduction

Organisms store excess energy and release it when they suffer from energy deprivation. For most eukaryotes, the preferred storage compounds are lipids in the form of triacylglycerols (TAGs). TAGs are non-polar and can be stored in a nearly anhydrous form. Their complete oxidation yields more than twice as much energy as protein or carbohydrate hydrolysis, on a per unit volume basis. TAGs are accumulated in specialised structures that have been named differently depending on the organism or the cell type studied. For example, they have been designated “lipid droplets” in adipocytes, “lipid particles” in yeast, or “oil bodies” or “oleosomes” in plants. However, these structures share common characteristics. They are usually 0.2 to 2µm in diameter and are composed of a hydrophobic core of TAGs surrounded by a monolayer of phospholipids coated with specific proteins [1].

In plants, the main site of TAG storage is in the embryo and/or endosperm tissues of the seeds, depending on the species [2]. When the seeds germinate, the TAGs are degraded in order to produce a carbon source that will fuel the embryo’s postgerminative growth and allow it to become a photosynthetically active seedling with a root system and leaves. The pathways involved in the conversion of TAGs to sugars in germinated seeds have been extensively studied and progress in our understanding of the process has recently been reviewed [3]. An overview of this process is shown in *figure 1*. Briefly, TAGs are hydrolysed to free fatty acids and glycerol by TAG lipases. The glycerol is phosphorylated and enters gluconeogenesis after its conversion to dihydroxyacetone phosphate [4]. The free fatty acids are transported to the glyoxysome where they are activated to acyl-CoAs and enter the ß-oxidation spiral. The acetyl-CoAs produced by ß-oxidation are channelled to the glyoxylate cycle and then to gluconeogenesis, which produces the sugars that sustain embryo growth following germination.

It is noteworthy that, in addition to postgerminative TAG breakdown, some degradation has also been observed during late seed development in several plant species and that this can account for a ~10 % decrease in TAG content in oilseed rape (*Brassica napus*) embryos by the time desiccation is complete [5]. It is not clear what the precise physiological role of this TAG breakdown is but it does potentially represent a significant yield loss that might be preventable in oilseed crops [5].

In this review, we briefly outline the synthesis of TAG and formation of oil bodies in seeds before focusing on the hydrolysis of TAG and the identity, role and regulation of the lipases that catalyze this process. Although we introduce some of the relevant biochemical and molecular literature concerning lipases in seeds we place most emphasis on the recent discovery of SUGAR-DEPENDENT1 (SDP1) and its role in lipolysis in *Arabidopsis thaliana* seeds.

2. Oil deposition in seeds

2.1. Triacylglycerol synthesis

TAGs consist of three fatty acids esterified to a glycerol backbone. TAG formation in developing seeds is catalysed by the enzymes of the Kennedy pathway in the endoplasmic reticulum (ER) [6]. Fatty acids are transferred from acyl-CoA to the glycerol-3-phosphate backbone at the *sn-1* and *sn-2* positions by two consecutive acyltransferase reactions yielding the central metabolite phosphatidic acid. Phosphatidic acid is then dephosphorylated to diacylglycerol (DAG) and a third fatty acid is transferred to the free *sn-3* position of DAG by diacylglycerol acyltransferases (DGAT; EC 2.3.1.20) [6]. Only this last step is specific to TAG biosynthesis and DAG is often converted to phosphatidylcholine (PC) and, to a lesser extend, phosphatidylethanolamine (PE) before being made available for TAG biosynthesis. The acyl chains in PC and PE are desaturated or otherwise modified before becoming available again for TAG synthesis [6]. An alternative mechanism for TAG biosynthesis has also been described more recently. A phospholipid: diacylglycerol acyltransferase (PDAT; EC 2.3.1.158) has been cloned and characterised in Arabidopsis. This enzyme allows the transfer of the *sn-2* acyl group from PC or PE to DAG, resulting in the formation of TAG [7]. At present the physiological importance of this route remains to be proven [8].

2.2. Oil body formation

Oil bodies are derived from the ER but the exact mechanism of their biogenesis is still in question. It has been proposed that TAGs accumulate within the intra-bilayer space of the ER membrane. Distension of the membrane at these sites causes the budding of TAG droplets surrounded by a phospholipids monolayer. Oil bodies then mature by becoming encircled by rough ER and by acquiring their coating proteins [1]. However, more recently, a new mechanism has been proposed for lipid body biogenesis. In mammalian macrophages, oil droplets appear to be formed in ER cups that possess clusters of the protein adipophilin in the cytoplasmic leaflet of the ER membrane. It has been suggested that adipophilin transfers lipids from the ER membrane to the droplet surface, forming the monolayer of phospholipids that encircles the TAG core [9]. In Arabidopsis seeds five different oleosins, a caleosin, a steroleosin, one putative aquaporin and a glycosylphosphatidylinositol-anchored protein have been found associated with the oil body surface [10]. Oleosins are the major structural proteins of plant oil bodies. They cover most of the oil body's surface and are responsible for preventing coalescence during seed maturation, desiccation and germination. Oleosins maintain small structures by providing steric hindrance and electronegative repulsion. The maintenance of a high surface-to-volume ratio is potentially important to ensure a sufficient surface area for lipase action and/or interaction with organelles such as peroxisomes [1, 2, 11].

3. Lipolysis in seeds

TAG lipases (EC: 3.1.1.3) hydrolyse TAGs at the oil/water interface to yield free fatty acids and glycerol. They have been characterized from numerous organisms and until recently all true lipases belonged to the super-family of α/ß fold hydrolases that contain a catalytic triad composed of Ser, His, and Asp or Glu [12]. The catalytic serine residue sits in a conserved motif made of five amino acids (GXSXG) [12]. In many lipases the active site is sheltered by a lid made of an α-helix stretch that is moved out upon the adsorption of the enzyme to the interface [13]. The search for lipases involved in TAG breakdown during the post-germinative growth of seeds started a long time ago but only recently has molecular identity and physiological proof of function been obtained for some of these genes.

3.1. Purification and cloning of candidate lipases

In the majority of cases where lipase activity has been studied in seeds it is only detectable upon germination and increases concomitantly with the disappearance of TAG [14]. Fractionation studies have shown that these lipase activities are often membrane associated and can be found in the oil body, peroxisome or microsomal fractions of seed extracts, depending upon the species [14, 15]. Since lipases are interfacial enzymes, those that reside on the surface of the oil body might logically be expected to play a role in TAG breakdown. However, studies using electron microscopy have indicated that oil bodies are in close proximity with other organelles (particularly peroxisomes), and it has long been hypothesized that an association between them might be required to facilitate fatty acid release and transfer [16-18]. In support of this we have recently identified a protein in the peroxisomal membrane that is required for oil body-associated lipase activity and TAG hydrolysis in germinated Arabidopsis seeds [18].

Lipases have been purified from the seeds of plants such as maize (*Zea mays*) [19], castor bean (*Ricinus communis*) [20, 21], oilseed rape [22] and ironweed (*Vernonia galamensis*) [23]. More recently, several genes have been cloned that encode proteins with TAG lipase activity from castor bean, Arabidopsis and tomato (*Solanum lycopersicum*) [24-26]. These cloned lipases are characteristic α/ß hydrolases and they are all capable of hydrolysing TAGs but not phospho- or galactolipids. The castor bean "acid" lipase was identified by proteomic analysis of oil body associated proteins and was consequently designated OIL BODY LIPASE 1 (OBL1) [24]. Whereas this protein is associated with purified oil bodies and is very abundant in seeds, its physiological role is unclear and it is unlikely to be related to the degradation of TAG upon germination [24]. OBL1 is probably inactive at physiological pH and it is most abundant prior to germination when little or no TAG breakdown is occurring [27]. The Arabidopsis *AtLIP1* and tomato *LeLID1* TAG lipases have been cloned by homology with previously characterised lipases. *LeLID1* is a tomato homolog of Arabidopsis *DEFECTIVE IN ANTHER DEHISCIENCE1 (DAD1)*. AtDAD1 catalyzes the liberation of free linolenic acid to serve as a substrate for jasmonate synthesis in flower organs [28]. AtDAD1 has been described as a phospholipase A_1. However, its homolog in tomato is capable of hydrolysing TAG [26]. The expression profile of *LeDAD1* is consistent with a role in TAG breakdown since its transcript abundance increases during germination and drops when the cotyledons open. However, there is no direct evidence that *LeDAD1* is involved in TAG mobilisation and the physiological role of *LeDAD1* in seeds still needs to be confirmed [26]. In Arabidopsis, AtLIP1 shares 30% amino acid identity to human gastric and human lysosomal acid lipases [25]. When expressed in *E. coli*, the recombinant protein displays an activity against TAG but not phospho- or galactolipids and it is active in a pH range between 4.0 and 7.0. The analysis of a T-DNA null mutant in *AtLIP1* has suggested that this enzyme is not required for TAG degradation in seeds and the precise physiological role of this gene also remains unknown [25].

3.2. Identification of SUGAR-DEPENDENT1 in Arabidopsis seeds

In 2006, the first lipase mutant that is impaired in seed TAG mobilisation was isolated using a forward genetic screen [29]. Disruption of genes involved in TAG utilisation in Arabidopsis leads to postgerminative growth arrest and this phenotype can be rescued by the addition of an alternative carbon source such as sucrose in the culture medium [16, 30]. An ethyl methanesulfonate-mutagenised seed population was screened for this "sugar-dependent" phenotype. Among the six new loci isolated using this screen, three were impaired in TAG hydrolysis upon germination [29]. The first mutant locus called *sugar-dependent1* (*sdp1*) (*figure 2A*) was mapped to chromosome 5 and the cloning of the wild type gene revealed that it encodes a patatin-like lipase, similar to mammalian Adipose Triacylglyceride Lipase (ATGL), *Saccharomyces cerevisiae* triacylglycerol lipase 3 (TGL3), TGL4 and TGL5 and *Drosophila melanogaster* Brummer [31-33]. Patatin-like TAG lipases (PTLs) are related to the calcium insensitive phospholipase A_2 (iPLA$_2$) family, of which potato (*Solanum tuberosum*) Patatin is the founding member [34]. Proteins from this family are serine esterases, like classical α/ß-hydrolases, but their folding topology is different and their catalytic mechanism relies on a Ser-Asp catalytic dyad [35]. SDP1 lipase activity is blocked by the iPLA$_2$ mechanism-based inhibitor [E]-6-[bromoethylene]-3-[1-naphthalenyl]-2H-tetrahydropyran-2-one suggesting that SDP1 also utilized this mechanism [29].

The phenotype of the *sdp1* mutant suggests that SDP1 plays a major role in TAG hydrolysis in germinated Arabidopsis seeds [29]. (i) *sdp1* mutants are defective specifically in lipase activity associated with the oil body membrane; (ii) five day old seedlings grown on sucrose retain clusters of oil bodies which would normally be degraded by this stage (*figure 2B*); (iii) whereas the level of TAG in wild type seedlings falls by 95% over the course of the first 5 days of postgerminative growth, the TAG level drops by only ~20% in *sdp1-1* seedlings (*figure 2C*). The small amount of TAG mobilization that still occurs in *sdp1* mutants, combined with the presence of protein and soluble carbohydrate reserves, probably explains why postgerminative growth of *sdp1* is retarded but not arrested [29]. Defects in peroxisomal fatty acid import, activation, and ß-oxidation can cause more severe postgerminative growth arrest phenotypes and in some cases dramatically reduce germination frequency [30]. It has been argued that these phenotypes are not entirely due to carbon shortage but to additional roles of peroxisomal ß-oxidation in germination [30]. Like Arabidopsis *sdp1*, the yeast *tgl3Δ* deletion strain also retains some capacity for TAG breakdown [36]. However, TAG hydrolysis is effectively blocked when a *tgl3Δ tgl4Δ tgl5Δ* triple mutant is created [31]. Arabidopsis contains one SDP1 homologue (SDP1-like) and a single protein that is distantly related to human ATGL (ATGL-like) (*figure 3A*). Public micro-array data suggests that these two genes are also expressed in seeds [29]. Therefore, they could account for the residual rate of TAG hydrolysis that occurs in germinated *sdp1* seedlings [29]. We have recently created a *sdp1 sdp1-like* double mutant and phenotypic analysis of this line confirms that SDP1L can account for most of the remaining TAG degradation that is observed in *sdp1* seedlings. Indeed the double mutant has a more severe phenotype with respect to postgerminative growth arrest and TAG breakdown is almost completely prevented (Quettier, Kelly and Eastmond unpublished data).

3.2.1. Sub-cellular localisation

The fact that oil body membranes from germinated *sdp1* seeds are deficient in lipase activity strongly suggests that SDP1 is an oil body associated protein. This hypothesis was also supported by analysing the sub-cellular localization of a SDP1-GFP (green fluorescent protein) fusion protein transiently expressed in cotyledons of Arabidopsis seedlings [29]. Localization to the oil body surface is consistent with a role in initiating TAG breakdown and all the other PTLs that have been characterised to date are also apparently associated with the oil body surface [31-33]. However, PTL targeting to the oil bodies is not fully understood. The major proteins responsible for oil body architecture in plants (oleosins) possess a long central hydrophobic domain that anchors them to the oil body surface [37]. By contrast PTLs don't possess such a domain. However, shorter hydrophobic stretches can be found in their sequences and these are believed to be responsible for the proteins' association with the oil body membrane [32, 36, 38]. This has been demonstrated for ATGL, which possesses a hydrophobic stretch in its C-terminal region that is outside of the patatin-like domain (PLD). Deletion of the C-terminal part of the protein led to the cytosolic localisation of the protein [38]. On the other hand, in vivo localisation of drosophila Brummer fused to GFP has revealed that the PLD and the Brummer box (a motif conserved in a number of PLD containing proteins) are responsible for the correct targeting of the protein. The PLD alone enables the targeting of the protein to the oil body membrane but the Brummer box is necessary for the normal distribution of the protein on the oil body surface, precisely, in islands at the interdroplet contact sites [32].

In plants, it has also been suggested that major oil body proteins like oleosin or caleosin might play a role in regulating lipolysis by facilitating or blocking lipase access to its substrate [1, 37]. Disruption of a caleosin gene has recently been reported to slow the rate of TAG breakdown in germinated Arabidopsis seeds [39]. In mammals, lipid droplet surface proteins called perilipins are known to play a role in regulating lipolysis [40]. In addition to their apparent role in protecting TAG from lipases under basal conditions, perilipins appear to coordinate the recruitment and/or activation of lipases under lipolytic conditions [40]. However, oil bodies from yeast have no structural proteins and yet TGL3, 4, and 5, as well as ATGL and SDP1, when expressed in yeast, can all associate with the oil body surface in vivo [31, 36, 41] (Eastmond unpublished data). In plants it has also been shown that various exogenously applied lipases can readily hydrolyse TAG from oil bodies that have been purified from seeds [42]. These data suggest that in plants structural proteins on the surface of the oil body might not play a significant role in governing lipolysis.

3.2.2. Substrate specificity

The catalytic properties of SDP1 are broadly consistent with those reported for the TAG lipase activity from oil body membranes of oilseed rape [43, 44]. This species is a relative of Arabidopsis. Both activities have a neutral to alkaline pH optima and can hydrolyze a range of TAGs containing long-chain saturated and unsaturated fatty acids. The lipase activity measured in purified oil body membranes from oilseed rape is able to hydrolyze TAG, DAG, and monoacylglycerol (MAG) [44]. By contrast, SDP1 exhibits specificity toward TAG and is significantly less active against DAG and almost inactive on MAG. These data support the hypothesis that SDP1 is responsible for the first hydrolytic attack on the TAG molecule in germinated Arabidopsis seeds. A similar preference for TAG has been reported for human ATGL and drosophila Brummer, as well as for yeast TGL4 [32, 33, 41]. By analogy with lipolysis in human adipocytes [33], it is possible that DAG and MAG lipases work in concert with SDP1 to hydrolyze TAG to fatty acids and glycerol (Fig. 1). These lipases may also be associated with the oil body membrane [44], and their identity remains to be resolved. It remains possible that SDP1 and SDP1L have different substrate specificity and that together these enzymes are able to completely degrade TAG in Arabidopsis seeds. In yeast TGL3 has a significant DAG lipase activity while TGL4 does not [41].

Plants synthesise a large variety of fatty acid backbones. More than 200 species have been reported and classified according to the arrangement of double bonds, triple bonds and other modifications [45]. Where a single molecular class of TAG predominates in the seed it appears that selective pressure has yielded a lipase with the appropriate specificity to hydrolyse that TAG following germination [46]. Among the most extreme examples of this typoselectivity appear to be the lipases from ironweed and *Cuphea procumbans*, which have been reported to exhibit more than 20-fold selectivity for trivernolin and tricaprin, respectively [23, 46]. Because PTL functions are remarkably conserved between animals, yeast and Arabidopsis, it is likely that PTLs are involved in TAG breakdown in other plant species, although this remains to be demonstrated. It would prove interesting to study the typoselectivity of PTLs from species that store "unusual" fatty acids in the form of TAG in order to better understand the basis of lipase typoselectivities.

3.2.3. Regulation

Because SDP1 catalyzes the initial step in TAG breakdown, it is a prime target for the regulation of this physiologically important process. Generally, very little lipase activity is detected in imbibed seeds prior to germination, and the activity increases dramatically following germination, coinciding with the onset of TAG mobilization [14, 25, 43]. Surprisingly, *SDP1* transcript levels do not correlate positively with enzyme activity since they are highest throughout seed maturation and in imbibed seeds transcript abundance actually appears to decline prior to radicle emergence [29]. This discrepancy could be explained if SDP1 were regulated at the posttranscriptional level. Biochemical studies on oil body lipase activity from germinated seeds have suggested that the enzymes could be subject to various forms of regulation. These include stimulation by Ca^{2+} [27], inhibition by acyl-CoAs (plus CoA) [47], and gibberellic acid induced relocation from the vacuole to the oil body [48]. To date, studies on Arabidopsis mutants lend some support for a feedback mechanism by which acyl-CoA utilization could control the rate of lipolysis. Mutants that are defective in peroxisomal fatty acid/acyl-CoA import, activation, or ß-oxidation accumulate long-chain acyl-CoAs following germination and retain fatty acids in the form of TAG oil, which appears to remain in oil bodies [16, 30].

One level of regulation for SDP1 could be its phosphorylation state. Indeed, the protein possesses conserved putative phosphorylation sites. ATGL and TGL4 are both phosphorylated [33, 41] and in the case of TGL4, the protein has been identified as a substrate for the cyclin-dependent protein kinase Cdc28/CDK1 [49]. Activity of SDP1 might also be regulated by its interaction with other proteins. This is the case for ATGL, which is activated by comparative gene identification 58 (CGI-58). CGI-58 is an α/ß-hydrolase fold containing protein but it is not an active hydrolase since the catalytic serine in the GXSXG motif is replaced by an asparagine. However, CGI-58 activates ATGL by direct protein-protein interaction [50]. In basal lipolysis conditions (non activated adipocytes), CGI-58 interacts with perilipin, which binds CGI-58 to the lipid droplets. Upon lipolysis stimulation, CGI-58 is released from perilipin and can then activate ATGL [40]. Both yeast and Arabidopsis contain homologues of CGI-58 (ICT1 and At4g24160, respectively). Like CGI-58, ICT1 is a protein of the α/ß-hydrolase family in which the catalytic motif appears to be mutated [51]. Both CGI-58 and ICT1 also possess an extra domain, characteristic of acyltransferases, composed of a His and an Asp, separated by 4 less conserved residues (HXXXXD) [51, 52]. This domain confers a lysophosphatidic acid (LPA) acyltransferase activity to both proteins. Consequently, CGI-58 and ICT1 appear to be involved in phospholipids biosynthesis [51, 52]. It is worth noting that there is currently no evidence that ICT1 is able to bind TGL3, 4 or 5. Consequently, ICT1 may not be involved in the regulation of lipolysis as GCI-58 is. Furthermore, ATGL activation and LPA acylation by CGI-58 can function independently [52]. The function of the Arabidopsis CGI-58 homologue (At4g24160) is unknown at present.

4. Conclusion

Recent work suggests that SDP1 is most likely to initiate TAG breakdown in Arabidopsis seeds [29]. Although the discovery of SDP1 sheds new light on the mechanism lipolysis in seeds there still remain many gaps in our knowledge of this process. SDP1 appears to preferentially hydrolyse TAG and it remains to be determined whether additional DAG and MAG lipases are involved in lipolysis. It is also unclear how SDP1 activity is regulated over the course of seed development, seed germination and postgerminative growth. SDP1 appears to work at the surface of the oil body to release fatty acids, which must then be transferred to the peroxisome for β-oxidation. How SDP1 associates with the oil body surface, accesses its substrate, and precisely how fatty acids are then transferred to the peroxisome is also unclear at present. Further work is required to answer these important questions.

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Legends to figures

Figure 1: A schematic diagram of triacylglycerols (TAG) breakdown following seed germination. The TAGs stored in the oil body are hydrolysed to fatty acids (FA) and glycerol (Gly) by the sequential action of one or more lipases (1, 2, 3). Gly is converted to dihydroxyacetone phosphate (DHAP) by the action of Gly kinase (4) and Gly-3-phosphate dehydrogenase (5). FAs are transported into the peroxisome where they are activated to acyl-CoAs (6) and enter the β-oxidation spiral (7). The acetyl-CoA that is produced is converted to organic acids such as oxaloacetate (OAA) by the glyoxylate cycle (8). OAA and DHAP are converted to sugars such as sucrose (Suc) by gluconeogenesis (9). Mit. is mitochondrion. Arrows with dotted lines denote pathways, several of which are non-linear.

Figure 2: Phenotype of the *sdp1* mutant. (A) Postgerminative growth arrest of *sdp1* seedlings grown for five days in the light on agar plates with half-strength MS salts. Bar = 1 cm. (B) Electron microscopy images of cotyledon cells from *sdp1-1* mutant seedlings showing retention of oil bodies (OB). Bar = 1 µm. (C) TAG content of *sdp1-1* mutant seedlings. For B and C seedlings were grown for five days in the light on agar plates with half-strength MS salts plus 1% (w/v) sucrose.

Figure 3: (A) Phylogenetic tree for patatin-like lipases. The phylogenetic tree was constructed with ClustalW software using alignments of the full length amino acid sequences. The GenBank identifier (gi) for each sequence, from the top to the bottom, is 32698724, 24664320, 118486599, 42571733, 113645287, 6322973, 74676509, 549643, 15237603, 15230231, 115456133 and 115440091. (B) A schematic representation of the Arabidopsis SDP1, yeast TGL3, human ATGL and the potato Patatin proteins showing the position of the patatin-like domain, which is highlighted by a shaded box and contains the conserved GXSXG motif surrounding the catalytic serine.

Figure(s)
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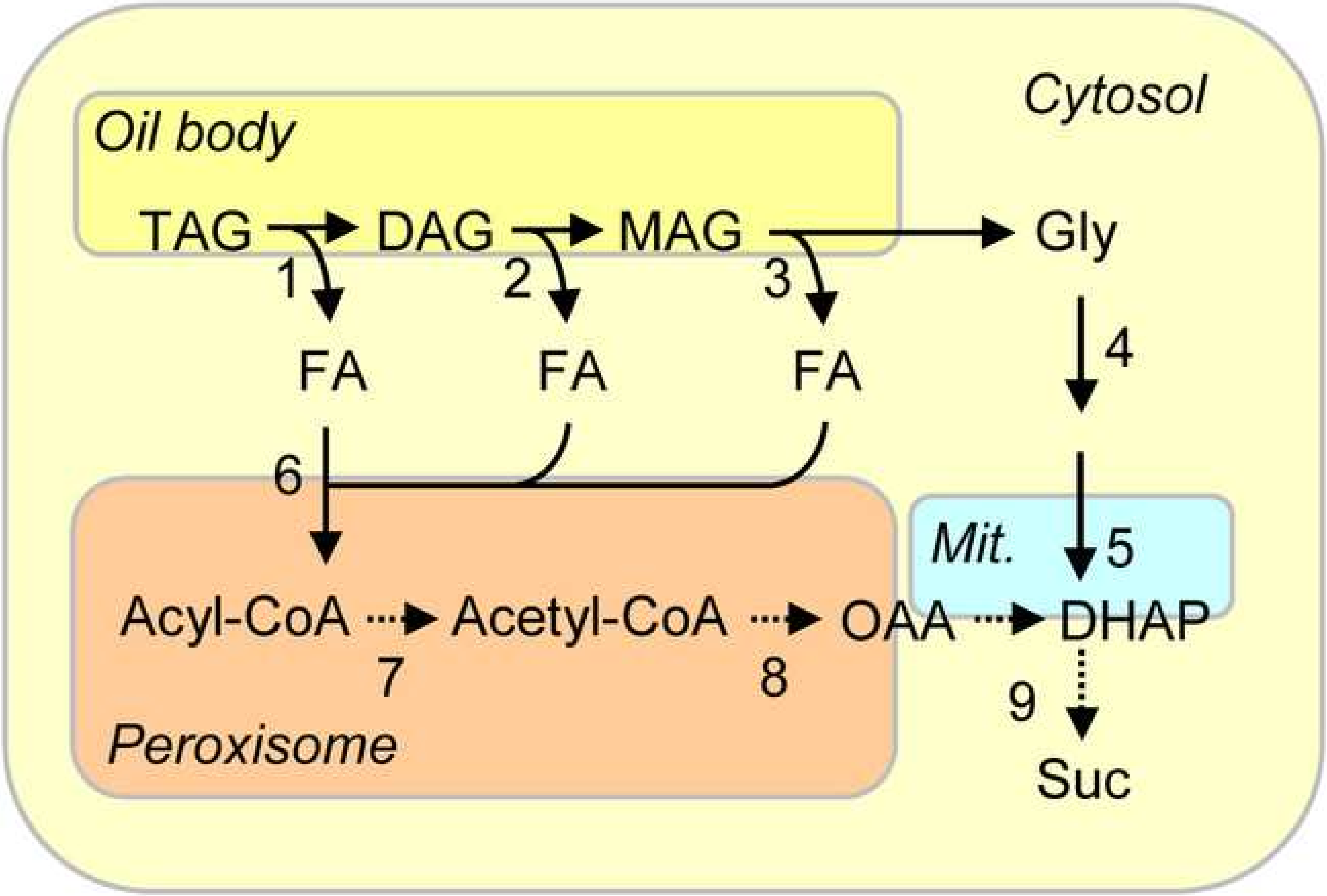
Cytosol
Oil body
TAG
DAG
MAG
Gly
1
2
3
FA
FA
FA
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Acyl-CoA
Acetyl-CoA
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Peroxisome
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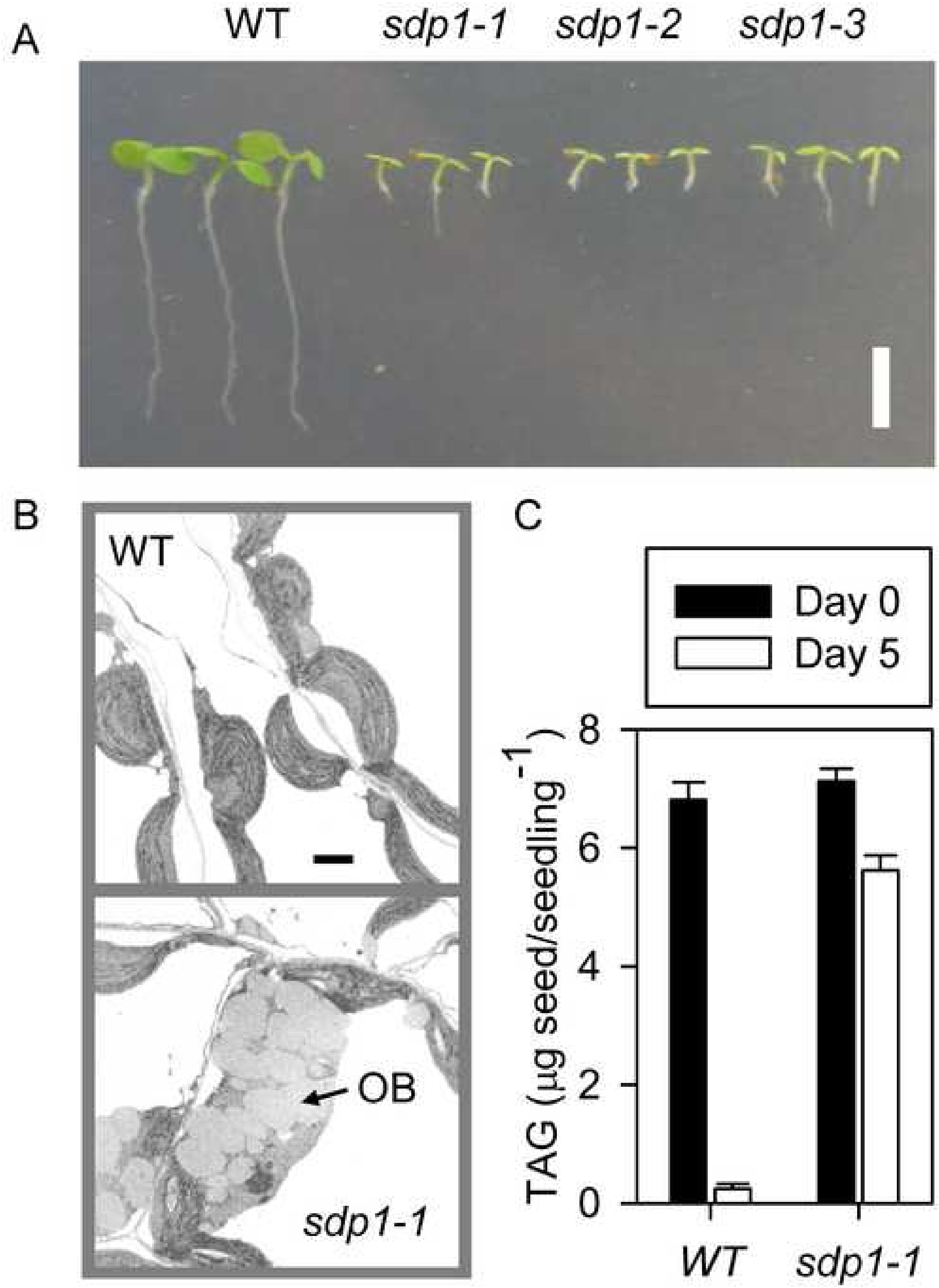

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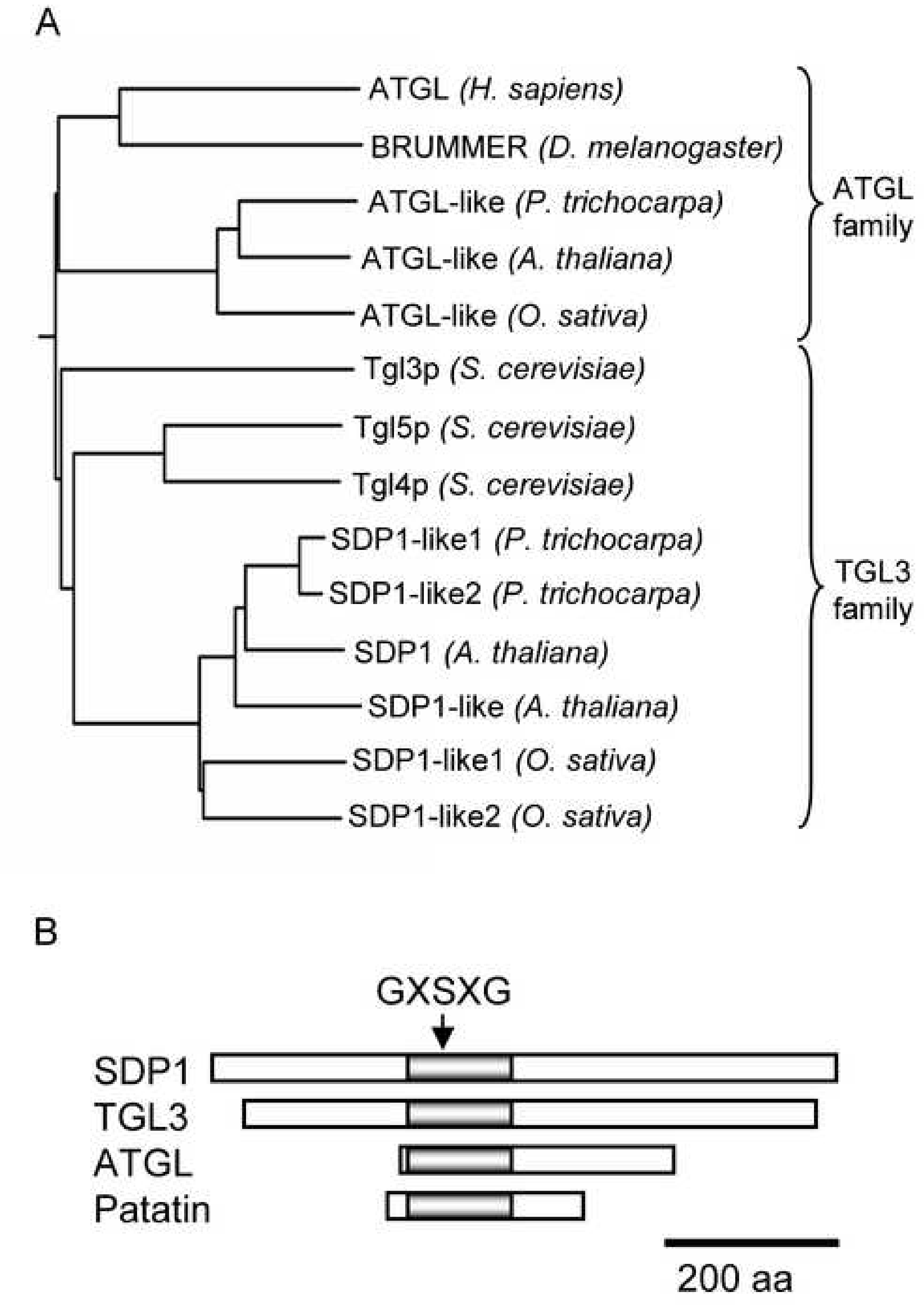
A
ATGL (*H. sapiens)*
BRUMMER *(D. melanogaster)*
ATGL-like *(P. trichocarpa)*
ATGL-like *(A. thaliana)*
ATGL-like *(O. sativa)*
Tgl3p *(S. cerevisiae)*
Tgl5p *(S. cerevisiae)*
Tgl4p *(S. cerevisiae)*
SDP1-like1 *(P. trichocarpa)*
SDP1-like2 *(P. trichocarpa)*
SDP1 *(A. thaliana)*
SDP1-like *(A. thaliana)*
SDP1-like1 *(O. sativa)*
SDP1-like2 *(O. sativa)*
ATGL family
TGL3 family
B
GXSXG
SDP1
TGL3
ATGL
Patatin
200 aa