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Lipid droplets degradation mechanisms from microalgae to mammals, a comparative overview

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ABSTRACT

Lipid droplets (LDs) are organelles composed of a hydrophobic core (mostly triacylglycerols and steryl esters) delineated by a lipid monolayer and found throughout the tree of life. LDs were seen for a long time as simple energy storage organelles but recent works highlighted their versatile roles in several fundamental cellular processes, particularly during stress response. LDs biogenesis occurs in the ER and their number and size can be dynamically regulated depending on their function, e.g. during development or stress. Understanding their biogenesis and degradation mechanisms is thus essential to better apprehend their roles. LDs degradation can occur in the cytosol by lipolysis or after their internalization into lytic compartments (e.g. vacuoles or lysosomes) using diverse mechanisms that depend on the considered organism, tissue, developmental stage or environmental condition. In this review, we summarize our current knowledge on the different LDs degradation pathways in several main phyla of model organisms, unicellular or pluricellular, photosynthetic or not (budding yeast, mammals, land plants and microalgae). We highlight the conservation of the main degradation pathways throughout evolution, but also the differences between organisms, or inside an organism between different organs. Finally, we discuss how this comparison can help to shed light on relationships between LDs degradation pathways and LDs functions.

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Abbreviations: AMPK, Adenosine monophosphate-activated protein kinase (AMPK); CE, cholesteryl ester; CMA, chaperone-mediated autophagy; DAG, diacylglycerol; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; FA, fatty acid; LD, Lipid droplet; MAG, monoacylglycerol; mTOR, mechanistic target of rapamycin; PtdIns(3)P, phosphatidylinositol 3-phosphate; PtdIns(4)P, phosphatidylinositol 4-phosphate; PRKA, protein kinase AMP-activated; TAG, triacylglycerol; SE, sterol ester; vCLIP, vacuole-LD contact site.

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1. Introduction

Lipid droplets (LDs) are found throughout the tree of life [1–3]. They contain a hydrophobic core, mostly composed of triacylglycerols (TAG) and steryl esters (SE), surrounded by a monolayer of membrane glycerolipids. Many proteins are found at their surface, either embedded in the lipid monolayer or associated through protein-lipid or protein-protein interactions [4,5]. For a long time, LDs were seen as simple storage organelles, used to absorb the excess of energy uptake (e.g. adipose tissues) or to provide energy to sustain developmental programs (e.g. plant seeds germination) [6,7]. This view has been progressively expanded to account for their central role in lipid metabolism and actual knowledge highlights the wide diversity of LD functions. In particular, LDs appear as essential in response to a multiple variety of stresses, of both abiotic and biotic origin, in algae and plants as well as in mammals and yeast [8–11]. However, these functions can vary greatly depending on the considered organism, tissue, environmental condition or even between LDs within the same cell.

In order to better understand LDs functions it is essential to apprehend their life cycle, from biogenesis to degradation. LDs biogenesis starts with the accumulation of neutral lipids between the two leaflets of the endoplasmic reticulum (ER) membrane; when their concentration increases, these neutral lipids coalesce into lenses and eventually bud from the ER, a process that can occur spontaneously but is promoted by proteins, in particular Seipin (reviewed in Refs. [12,13]). The Seipin protein functions, controlling the ER-LD junction and the TAG flux from ER to LDs, appear conserved in all eukaryotic cells (reviewed in Ref. [14]). Yet interactions of Seipin with other proteins emerge as potential important factors to modulate its activity and the composition of newly formed LDs [14,15].

Following their biogenesis, LDs fate can vary greatly. In oleaginous dry seeds, and to a lesser extent in adipocytes, which serve as storage, LDs are relatively stable organelles. By contrast, in unicellular organisms, and in most tissues from plants and animals, LDs can be very dynamic, and their number and size can quickly vary to respond to environmental cues (e.g. stress) or to developmental programs that trigger their formation or responsive remobilization. To answer these rapid LDs turnovers and their specific functions, two main degradation pathways have evolved in all eukaryotic groups: lipolysis and lipophagy [8,16–19]. The relative contributions of these pathways are difficult to estimate as they can vary depending on organism, tissue and condition considered. Moreover, while lipolysis has been studied for a long time, lipophagy studies are more recent, which may skew our understanding of LDs degradation. We will aim in the present review not to be exhaustive but to describe the different degradation pathways, highlighting their conservation throughout evolution, but also the differences between organisms, or inside an organism between different organs. Finally we will reflect on how LDs degradation relates to the functions of LDs. To this aim, we will focus on very different model

organisms: the budding yeast *Saccharomyces cerevisiae*, mammals for animal models, angiosperms with the major model *Arabidopsis thaliana* and finally microalgae with two very different groups, green algae, in particular *Chlamydomonas reinhardtii*, and stramenopiles, with a focus on diatoms, in particular *Phaeodactylum tricornutum*.

2. LD degradation through lipolysis

The first way to degrade LDs is to degrade their hydrophobic core, mainly composed of TAG and SE, through lipolysis [17]. Three main steps can be distinguished (Fig. 1): 1) removal or remodeling of the LDs protein coat, 2) lipid degradation (lipolysis *sensu stricto*) and 3) subsequent degradation of released fatty acids (FA) in the peroxisome and/or mitochondria. In this section, we will focus on the central step, that is SE and TAG hydrolysis, while the other steps will be discussed in section 3.

2.1. Steryl esters degradation

Several enzymes involved in SE degradation have been identified in yeast, namely Tgl1, Yeh1, and Yeh2, but their study suggests that SE degradation is mainly important for sterol homeostasis with little impact on the global dynamics of LDs ([20–23], reviewed in Ref. [24]). Enzymes involved in the breakdown of plant SE are still unknown, although the LD-ASSOCIATED LIPASE (LIDL) 1 and 2, identified by quantitative proteomic analysis of LD-enriched fractions derived from *A. thaliana* seedlings, are homologs of Tgl1 and have been hypothesized to function as sterol esterases [25]. In mammals, SE exist as cholesteryl-esters (CE) and specific enzymes involved in their breakdown have been identified. Ghosh et al. [26] described a neutral cholesteryl ester hydrolase (CEH) in human macrophages named CES1, that is located at the surface of LDs and mobilizes LD-contained CE [27]. CES1 overexpression results in an increased rate of cholesterol efflux to ApoA1, HDL (high density lipoproteins) and serum [28], suggesting that CES1 can regulate both the CE content of LDs and the cholesterol efflux. KIAA1363/NCEH1/AADACL1 has also been identified as a CE hydrolase as its knock-out (KO) in mice results in a decreased CE activity [29], while its overexpression in macrophages markedly decreases the CE content [30]. However, its implication in macrophage CE metabolism is debated, and other yet unidentified CEH may exist [31]. In general, the importance of CE hydrolases in LDs degradation remains controversial (reviewed in Ref. [17]).

2.2. TAG degradation

2.2.1. Canonical TAG degradation

Three main lipolysis steps are required for TAG degradation: to diacylglycerol (DAG), monoacylglycerol (MAG) and finally glycerol, with fatty acids (FA) released at each step (Fig. 1).

In mammals, the primary step of TAG hydrolysis to DAG is

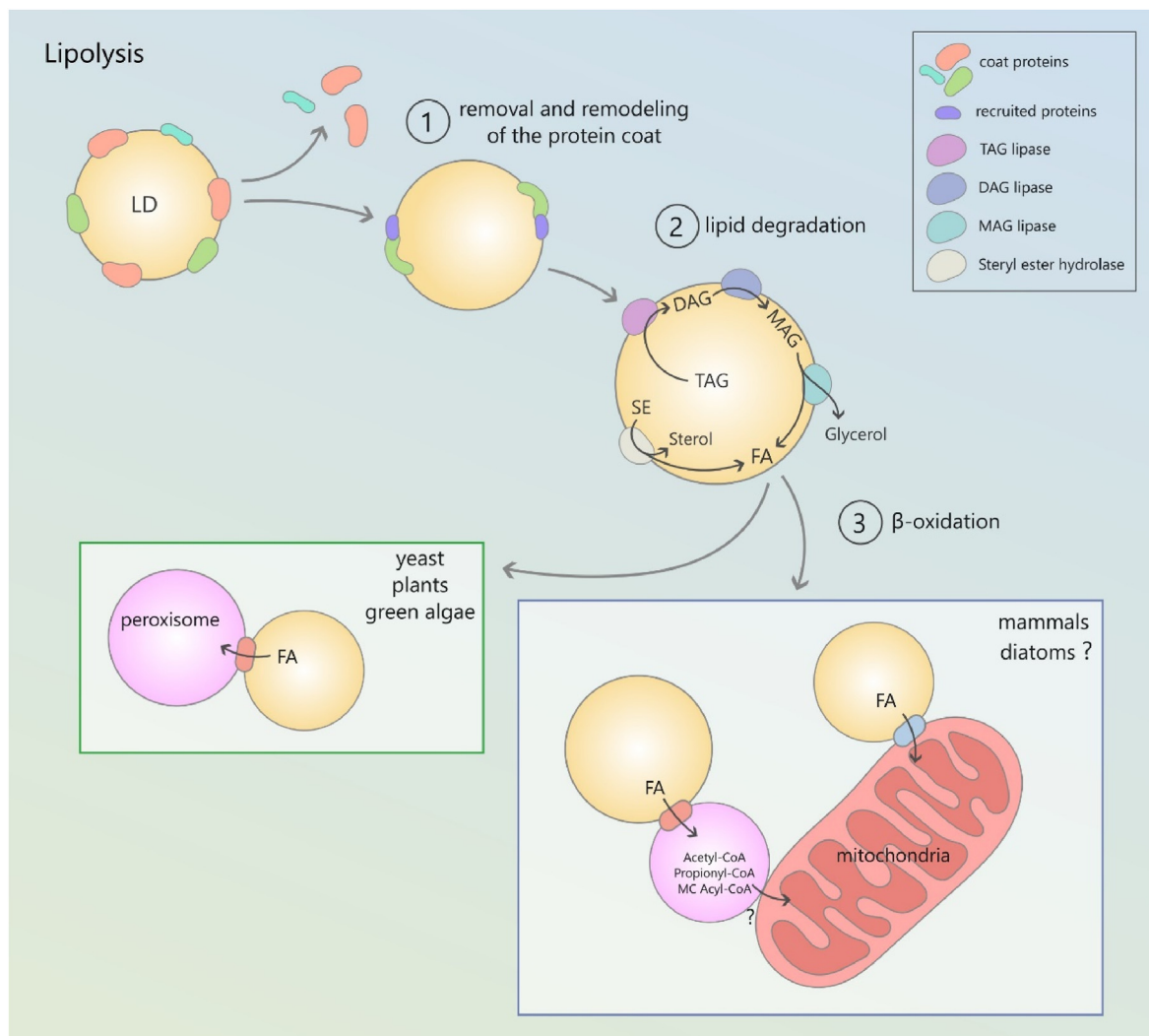


Fig. 1. Schematic representation of lipolysis mechanism. 1) Remodeling of the protein coat surrounding the LD that can include removal of proteins (e.g. oleosins in seeds, Perilipin2 and 3 in mammals) or post-translational modifications (e.g. Perilipin1 and 5 in mammals). This remodeling can induce recruitment of additional factors to the LDs; 2) Degradation of triacylglycerol (TAG) into diacylglycerol (DAG) and then monoacylglycerol (MAG) by membrane-localized enzymes, eventually leads to the release of glycerol and fatty acids (FA). A minor contribution is given by the degradation of sterol esters (SE). 3) FA can subsequently be degraded by β -oxidation, which can occur in the peroxisome, mitochondria or both depending on the considered organism.

catalyzed by the adipose triglyceride lipase (ATGL) [32], encoded by the *PNPLA2* (*patatin-like phospholipase domain-containing protein 2*) gene in humans (Table 1). KO of ATGL in mice results in a drastic decrease of lipolysis both in whole-body and specific adipocytes [33]. Accordingly, human mutations that disrupt ATGL activity are associated with decreased rates of glycerol and FA formation in response to lipolytic agents as well as with neutral lipid storage disease and myopathy [34]. Homologs of ATGL in other organisms have been widely identified and their function as major TAG lipases has also been demonstrated (Table 1). TGL4 is identified as the yeast ATGL homolog [35], but other patatin domain containing lipases have been identified. The major TAG lipase activity is ensured by TGL3, which shows unspecific activity towards TAG or even DAG [36]. TGL4 on the other hand shows a preference for TAG containing C14 and C16 acyl-chains, while a third enzyme, TGL5, is involved in the breakdown of TAG containing very long chain fatty acids C26 [35]. Both TGL3 and TGL5 also exhibit acyl-transferase activities, respectively towards lyso-phosphatidylethanolamine (LPE) and lyso-phosphatidic acid (LPA) [37]. In plants, the patatin-like lipase SUGAR DEPENDENT1 (SDP1) was identified in *Arabidopsis thaliana*

as the major lipase involved in TAG mobilization during seed germination [38], and a second homolog named SDP1-LIKE (SDP1L) was later identified [39,40]. Together, SDP1 and SDP1L contribute to over 95 % of TAG hydrolysis activity during seed germination [39], and SDP1 also appears to be important for LD turnover in vegetative tissues, in particular roots and stems [41]. Identification of lipases in microalgae is more recent. LISDP1 was identified in the LD proteome of the green alga *Lobosphaera incisa* [42]. More recently, LIP4 was identified in the green alga *Chlamydomonas reinhardtii* as a homolog of SDP1, with which it shares 44 % amino acid identity; LIP4 is involved in TAG remobilization during nitrogen starvation recovery as well as in TAG global homeostasis [43]. Similarly, SDP1 homologs have been identified in secondary endosymbionts, but appear to have different importance in TAG breakdown. Indeed, while in the diatom *Phaeodactylum tricorutum*, TGL1 seems to play a major role, similar to ATGL or SDP1 [44], its role seems much more limited in *Fistulifera solaris* [45]; additionally, study of NoTGL1 and NoTGL2 in *Nannochloropsis oceanica* shows that these ER-localized enzymes modulate TAG synthesis at the ER by degrading *de novo* synthesized TAG [46].

Table 1

Summary of the proteins shown to play a role in lipid droplet degradation by lipolysis in *Homo sapiens*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*.

	Name	Function	References
Lipases			
<i>H. sapiens</i>	ATGL	TAG lipase	[32]
	HSL	Hormone-sensitive lipase recruited at LDs surface upon cAMP stimulation, degrades preferentially DAG and cholesterol esters	[47]
	PNLPA3	TAG and DAG lipase	[48]
	MGL	MAG lipase	[49]
	CE1	TAG lipase and cholesteryl-esterase activities	[26,27]
<i>S. cerevisiae</i>	TGL4	TAG lipase	[35]
	TGL3	TAG and MAG lipase + acyltransferase (LPE)	[36]
	TGL5	TAG lipase + acyltransferase (LPA)	[35]
	Ldh1	TAG lipase, global lipid homeostasis	[50,51]
	Ayr1	TAG lipase	[52]
	Yju3	MAG lipase	[53]
	Yeh1 and 2	Sterol-esters hydrolases	[20]
	Tgl1	Sterol-esters hydrolases	[20,54]
<i>A. thaliana</i>	SDP1	TAG lipase, essential for seed germination	[41]
	SDP1-LIKE (SDP1L)	TAG lipase; overlapping function with SDP1 during seed germination, specific in the roots and leaves of mature plants	[39]
	AtOBL1	TAG, DAG and MAG lipase located at LDs surface; important for pollen tube growth	[55]
	MAGL8	MAG lipase associated with LDs in germinating seeds and in leaves	[56]
	At4g18550/ATDSEL LIDL1 and LIDL2	DAG and MAG lipase; involved in the regulation of storage oil mobilization and the establishment of early seedlings Putative sterol-esters hydrolases; located at LDs	[57] [25]
<i>C. reinhardtii</i>	LIP4	TAG lipase	[43]
	LIP1	DAG lipase	[58]
<i>P. tricornutum</i>	TGL1	TAG lipase	[44]
Coat proteins and coat proteins remodeling			
<i>H. sapiens</i>	PLIN1	LD coat protein removed through phosphorylation upon induction of LDs degradation	[59]
	PLIN2-3	LD coat proteins degraded through chaperone-mediated autophagy upon induction of LDs degradation	[60]
<i>A. thaliana</i>	OLE1-5	Oleosins, need to be removed for TAG degradation	[61,62]
	MIEL1, PUX10 and CDC48A	Oleosins degradation	[63–65]
<i>C. reinhardtii</i>	MLDP	LD coat protein, possible role in control of lipolysis	[66]
<i>P. tricornutum</i>	StLDP	LD coat protein, possible role in control of lipolysis	[67]
Lipolysis regulators			
<i>H. Sapiens</i>	CGI-58	ATGL activator	[68]
<i>S. cerevisiae</i>	Lct1	CGI-58 homolog	[69]
	Tld1	Negative regulator of Tgl3	[70]
<i>A. thaliana</i>	VPS29, PEX11e and FREE1	Regulate the translocation of SDP1 from peroxisomes to LDs through peroxisomal tubular extensions	[71,72]
	CGI-58	Regulates TAG breakdown; interacts with PXA1 at peroxisome surface	[73]
<i>C. reinhardtii</i>	CHT7	Major regulator of TAG remobilization during quiescence exit upon nitrogen resupply	[74]
	DTH1	regulator of TAG degradation, located at the LD	[75]
	LACS3	regulator of TAG degradation, located at the peroxisome	[76]
<i>P. tricornutum</i>	CGI-58	Located to mitochondria and promotes <i>de novo</i> fatty acid synthesis and TAG degradation through mitochondrial β -oxidation	[77]

Subsequent steps including DAG hydrolysis to MAG and MAG hydrolysis to glycerol and FA have been less well characterized. In mammals, the hormone-sensitive lipase (HSL, encoded by the *LIPE* gene) is the major DAG lipase but it also has a high activity in the hydrolysis of CE, and a lower one towards TAG, MAG, retinyl esters and short-chain acyl esters ([78], reviewed in Ref. [79]). Consistently, whole-body deficiency in HSL leads to DAG accumulation in the adipose tissue as well as decreased lipolysis in mice [80] and human [81]. Monoglyceride lipase (MGL) then catalyzes the hydrolysis of MAG, and can use both *sn*-1 MAG and *sn*-2 MAG, in which FA are esterified to the terminal or middle hydroxyl group of the glycerol backbone, respectively, as substrates [82,83]. In yeast, Yju3 has been identified as the functional ortholog of MGL [53]. In higher plants, several enzymes with DAG/MAG lipase activities have been identified, with different specificities [55–57,84]. In

particular, in *A. thaliana*, a cytosolic member of the DAD1-like acyl-hydrolase family encoded by At4g18550 demonstrates a specific ability to hydrolyze DAG and MAG at the *sn*-1 position and is crucial in the establishment of early seedlings [57]. The MAG lipase AtMAGL8 plays an important role in seeds and exhibits a notable substrate preference for MAG containing C20:1 fatty acids [56]. Finally in microalgae, few reports of DAG or MAG lipases exist, apart from the non-selective DAG lipase CrLIP1 in *C. reinhardtii* [58]. The development of bioinformatics tools has however allowed the identification of many new potential lipases that remain to be investigated [85,86].

2.2.2. Alternative enzymes and tissue specificities

In multicellular organisms, the role of lipases can vary from one tissue to another. In land plants, studies on LDs were primarily

focused on seeds germination but such interest has since expanded to other tissues, in particular the pollen tube, where LDs are essential, and leaves, where LDs play roles in response to various abiotic and biotic stresses (for review: [9,87]). Oil body lipase 1 (AtOBL1 in *A. thaliana*) has been identified as a TAG lipase, but can also degrade DAG and MAG with no obvious substrate specificity. Its role in seeds is very minor, but it is essential in pollen tubes [55]. Alternatively to lipases, a LD-specific lipoxygenase (LOX)-dependent pathway for lipid mobilization during the early stages of seed germination has been proposed in various plant species, such as *Arabidopsis*, watermelon, cucumber, wheat, barley, potato, tomato, sunflower and tobacco. This pathway allows direct oxygenation of linoleate residues directly on TAG and the coupling of TAG degradation with the generation of oxylipins, involved in signaling, or phytoalexins [88,89]. In mammals, the canonical pathway through ATGL and HSL accounts for over 90 % of TAG degradation in adipocytes [90,90]. Many other lipases have been identified, but their importance even in other tissues remains poorly evaluated (reviewed in Ref. [17]). For instance, PNLPA3, a close relative of ATGL that can hydrolyze TAG and DAG [48], is expressed in both white and brown adipose tissue in mice, is highly abundant in human hepatocytes and is associated with liver diseases; however, PNLPA3 KO has little impact and deleterious effects are observed as results of dominant negative mutations that increase its association with comparative gene identification 58 (CGI-58) to the detriment of ATGL association and activation [91–93] (cf. sections 3.1 and 3.2.2).

3. Lipolysis control mechanisms

Lipolysis requires tight control to ensure that LDs breakdown answers to the cell/organism needs at a given time and/or in response to external or internal cues. As shown on Fig. 1, lipolysis involves three main steps, all of which are regulated through different mechanisms.

3.1. Removal or remodeling of the lipid coat

In plant seeds, LDs are very important to ensure proper germination and the establishment of the developing seedling, thus regulating the precise timing and sequence of LDs degradation is of high importance. Plant seed LDs are covered by specific proteins called Oleosins [7,94], which can protect stored TAG from lipolytic enzymes [61,62] and are absent from LDs in vegetative tissues. The removal of Oleosins is tightly regulated through initial ubiquitination and subsequent proteasome degradation [95,96]. It involves the sequential recruitment of MYB30-interacting E3 ligase 1 (MIEL1), plant ubiquitin regulatory X (UBX) domain-containing protein10 (PUX10) and cell division cycle 48A (CDC48A) ATPase [63–65] (Table 1). However, protection of TAG by Oleosins is not tight-proofed [97] and additional mechanisms are required to regulate lipolysis.

Similarly to Oleosins, Perilipins (PLIN), the major proteins found at the surface of LDs [98] in mammals, participate in lipolysis regulation ([99], reviewed in Ref. [100]). PLIN2 and 3 are degraded by another mechanism called chaperone-mediated autophagy (CMA): *in vivo* studies revealed that CMA degradation of both proteins is increased during starvation, and that their degradation enhances LDs degradation through lipolysis, but also through macroautophagy (see section 4) [60] (Table 1).

Yet, protein coat removal is not a general rule. PLIN1, the major coat protein in white adipocytes, can be phosphorylated by protein kinase A (PKA), which is activated by cyclic Adenosyl MonoPhosphate (cAMP) signaling in response to β -adrenergic stimulation (reviewed in Ref. [59]). This phosphorylation triggers the

dissociation of PLIN1 and the major ATGL activator CGI-58 ([68] reviewed in Ref. [101]). A similar mechanism has been shown for PLIN5, which is important in oxidative tissues (heart, skeletal muscle, liver, brown adipose tissue) ([102–104], reviewed in Ref. [100]). In land plants, Lipid droplet associated protein (LDAP) and LDAP-interacting protein (LDIP) are the major LDs proteins in vegetative tissues, where they play important roles for LDs homeostasis [105–107] but their potential involvement in LDs degradation remains to investigate. Finally, in the diatom *Phaeodactylum tricornutum*, Stramenopile Lipid Droplet (StLDP) protein, which is the main coat protein [108–110], seems required for proper LDs degradation [67], although the mechanism remains unknown.

3.2. Regulation of lipolytic enzymes activity

3.2.1. In unicellular organisms

In unicellular organisms, cell cycle appears as a major regulator of metabolism and particularly of lipolysis. Indeed, in budding yeast, the TAG synthesis enzyme Pah1 is inhibited [111,112] while the TAG lipase TGL4 is activated [113] by Cdk1/Cdc28 during log phase. This regulation is consistent with the central role of TAG in lipid metabolism, as a source of building blocks for membrane lipids [114]. In photosynthetic microorganisms, light is another essential growth factor and circadian rhythms play important roles in the regulation of the cell cycle and of energy metabolism. This has been well shown in the diatom *P. tricornutum* [115–118], where LDs form during the day and are degraded during the night, when cell division occurs. Yet this coordination does not imply direct regulation of TAG degradation by cell-cycle dependent mechanisms. Such regulation has only been shown so far in the green alga *Chlamydomonas reinhardtii*, in which the regulatory protein COMPROMISED IN TAG HYDROLYSIS7 (CHT7) [74], involved in the exit of quiescence following nitrogen resupply, appears as a major regulator of TAG remobilization, linking the resumption of cell cycle and TAG hydrolysis.

This last examples also shows the importance of lipolysis following nutrient replenishment in microalgae. Another protein, Delayed in Triacylglycerol Hydrolysis (DTH1), which has only been identified in Chlorophyceae, localizes to the LDs and is a major player in lipolysis following nitrogen resupply in *C. reinhardtii*; however, although it is associated with the early steps of LDs degradation, upstream of β -oxidation, its exact function remains elusive [75].

Finally, finer regulation can also occur in unicellular organisms, for example to degrade specific LD subtypes. In budding yeast, it has recently been shown that the TG-associated LD protein 1 (Tld1) protein localizes specifically to LDs containing TAG but not SE and can specifically inhibit their degradation by TGL3 [70].

3.2.2. In multicellular organisms

In mammals, hormonal signals control LDs lipolysis through different mechanisms (reviewed in Ref. [17]). Both ATGL and HSL are regulated at the transcriptional level and can be upregulated in response to endocrine, paracrine or autocrine factors including hormones, cytokines and neurotransmitters. Peroxisome proliferator-activated receptor γ (PPAR γ) in particular can promote lipase expression in response to starvation [119,120]. HSL expression can also be activated by sterol regulatory element binding proteins (SREBPs) [121]. In addition to this transcriptional regulation, the activity of lipases is regulated post-translationally, both directly and indirectly through cyclic Adenosyl MonoPhosphate (cAMP) signaling. Indeed, protein kinase A (PKA), which is activated by cAMP in response to β -adrenergic stimulation, can phosphorylate HSL in addition to PLIN1 (cf. section 3.1). This phosphorylation

triggers the relocation of HSL to LDs as well as activation of ATGL by CGI-58, that follows CGI-58 and PLIN1 dissociation ([68] reviewed in Ref. [101]). But ATGL can also be regulated by intracellular glucose levels through polyubiquitination, as glucose depletion leads to decreased assembly of the Golgi-located E3-ligase complex CUL7^{FBXW8}, reducing ATGL polyubiquitination, which increases its stability and enhances lipolysis [122].

In plant seeds, the main lipase SDP1 is initially localized on peroxisomes. Its relocalization to LDs requires the formation of peroxisomal tubulations called peroxules [71,123]. Recent advances have shown involvement of the endosomal sorting complex required for transport (ESCRT) machinery and PEROXIN 11e (PEX11e) in the formation of these tubules [72] but the triggering mechanisms remain elusive. Germination defects in SDP1 mutants can be rescued by providing sugar, hence the protein's name [38], but, like in mammals, sugar levels can modulate SDP1 activity, although the mechanism is very different. Indeed, sucrose supplementation inhibits the interactions between peroxisome and LDs, and thus the lipolytic activity of SDP1 [123].

3.3. Interactions with mitochondria and/or peroxisome and subsequent fate of FA

The peroxisomal localization of SDP1 in higher plants provides a direct link between lipolysis *sensu stricto* and further degradation of FA by β -oxidation, which occurs in the peroxisome in most organisms, but also in mitochondria in animals and diatoms (Fig. 1). Direct interactions between LDs and FA degradation organelles appear as an important way to regulate the fate of FA released through lipolysis.

In mammals, mitochondrial β -oxidation appears as the major pathway, feeding ATP-synthesis [124], while the peroxisome is responsible for the degradation of very-long chain fatty acids (VLCFA) and participate in cellular thermogenesis [125]. Many proteins participating in contact sites between LDs and these 2 organelles have been described (for review: [126]). In particular, both PLIN 2 [127] and PLIN5 [128] have recently been shown to participate in the tethering of LDs and mitochondria to promote FA β -oxidation following energy deprivation. Another LD protein, Spastin, participates in the tethering of LDs and peroxisome; as for SDP1 relocalization, this association involves ESCRT components [129]. A direct link between lipolysis and peroxisome is also provided by the role of PEX5 in recruiting ATGL to LDs upon fasting [130]. As these different systems have been investigated and shown in different cell types, it remains to investigate whether they can act in synergy or reflect different strategies allowing to fulfill specific needs of cells.

Proteins tethering LDs and β -oxidation organelles are not known in microalgae. Yet, some proteins linking lipolysis and β -oxidation have been identified. We have previously mentioned the importance of CGI-58 in regulating ATGL in mammals (see sections 3.1 and 3.2.2). CGI-58 homologs have been identified in *A. thaliana* [131], yeast [69] and microalgae [77,132] but their localizations and functions differ. Ict1, the yeast homolog of CGI-58, was originally described as an Acyl-CoA-dependent lysophosphatidic acid acyltransferase [133]. In *A. thaliana*, CGI-58 function in TAG breakdown is conserved [39,131] but unlike its mammalian counterparts, it localizes to peroxisome and interacts with the peroxisomal ABC transporter (PXA1) [73]. Plant CGI-58 seems to play only a minor role in seeds LDs breakdown, but its loss causes a major effect in plant leaves [131]. In diatoms, CGI-58 functions seem to diverge. Indeed, while reduction of CGI-58 homolog levels in the diatom *Thalassiosira pseudonana* has the expected effect of reducing TAG hydrolysis [132], the overexpression of the *P. tricorntutum* homolog also leads to an overaccumulation of TAG; moreover, CGI-58 is

reported to be localized to mitochondria and the authors suggest that it plays a role in promoting *de novo* fatty acids synthesis as well as TAG degradation through mitochondrial β -oxidation [77]. The localization and function of CGI-58 homologs in plants and diatoms suggests that these proteins could participate in tethering LDs and respectively peroxisome and mitochondria, though such role remains to be investigated.

Finally, in the green alga *C. reinhardtii*, the long chain acyl-coA synthetase 3 (LACS3), which is located in the peroxisome and links TAG degradation and peroxisomal FA β -oxidation could be an interesting candidate for tethering of LDs and peroxisome [76].

4. Lipophagy

Autophagy is a general process to target the degradation of specific organelles or compounds (for review: [134,135]). Initially described in yeast and mammals, autophagy core components are well conserved in plants (for reviews, see Refs. [136–138]) and algae (for review [139]). In plants, autophagy plays an important role in response to biotic and abiotic stresses. In agreement, plants having a decrease in autophagy flux usually present a higher sensitivity to many stresses including heat, drought and nutrient stresses, likely due to the role of this process in cell compounds recycling and damaged or unwanted organelles removal under harsh environments (for reviews, see Refs. [137,140]). The link between autophagy and LDs is complex as autophagy can be involved in both LDs biogenesis and degradation and LDs can also play a role in autophagy by providing lipids for autophagosome biogenesis (for review: [141]). Here we will focus on the role of autophagy in LDs degradation.

Regulation of lipid metabolism through autophagy, also known as lipophagy, has only been shown quite recently [142,143]. Several autophagy, and subsequently lipophagy, pathways can be distinguished. Macro- and micro-autophagy were initially described based on electronic microscopy observations (for review: [144]). Macroautophagy has been known for a long time [145]. It involves membrane elongation and the formation of an autophagosome, that will engulf the targeted organelle, and fuse with lysosomes in mammals, or vacuoles in other organisms, to direct their degradation. By contrast, microautophagy involves direct contact of the targeted organelle with lysosomes or lytic vacuoles and their subsequent engulfment in these lytic compartments. The use of these two pathways for LDs degradation differs between organisms. While macrolipophagy seems to be a major process in mammalian cells, LDs degradation by different microlipophagy processes has been described in yeast, plants and microalgae. It should be however noted that lipophagy processes only start to be studied in some organisms and that the current view is probably still very partial.

4.1. Macrolipophagy in mammals

In mammals, macrolipophagy, also known as lysosome-mediated acid lipolysis, has been well described in the liver, where it is important to control intracellular lipid storage, free lipids and energy homeostasis (Fig. 2A) (for review: [146]). It involves the Lysosomal acid lipase (LAL) [147], encoded by the *Lipa* gene. While LAL is ubiquitously expressed, its highest expression is in macrophages and hepatocytes. LAL can degrade most components of LDs, including TAG and DAG but also steryl and retinyl esters ([148–150] reviewed in Ref. [17]). LDs degradation by LAL follows targeting of LDs to lysosomes through the classical macroautophagy route, that relies on the evolutionarily conserved autophagy-related genes Atg [151]. It is triggered in response to starvation *via* several signaling pathways, including repression of

the mTOR (mechanistic target of rapamycin kinase) complex 1 (MTORC1) [152,153], and activation of protein kinase AMP-activated catalytic subunit (PRKA)/adenosine monophosphate-activated protein kinase (AMPK) [154,155]. These in turn activate the ULK1/Atg1 (unc-51 like autophagy activating kinase 1) kinase complex [156,157], leading to the formation of a phagophore, which will evolve in forming the nascent autophagosome [158]. Specifically, the ULK1 complex phosphorylates and activates the class III phosphatidylinositol 3-kinase (PtdIns3K) complex, composed of Beclin-1/Vps30/Atg6, PIK3R4/VPS15, Atg14, PIK3C3/VPS34, AMBRA1 and NRBF2 [159]. Recruitment of this complex and production of a pool of phosphatidylinositol 3-phosphate (PtdIns(3)P) at ER extensions called omegasomes promotes the recruitment of PtdIns3P-binding proteins, such as ZFYVE1/DFCP1 (zinc finger FYVE-type containing 1/double FYVE domain – containing protein 1) [160] and WIPI2 (WD repeat domain, phosphoinositide interacting) protein [161]. WIPI2/Atg18 will then recruit and promote the assembly of the Atg12-5-16L1 complex [162] which acts as an E3-like ligase and recruits the E2-like protein Atg3 for the conjugation of the members of the MAP1LC3/LC3 (microtubule associated protein 1 light chain 3), also called Atg8, protein family to phosphatidylethanolamine (PE) [163]. LC3 proteins are important for autophagic cargo selection, phagophore elongation and closure. Last, autophagosomes appear to traffic along microtubules in higher eukaryotes, eventually targeting and fusing with lysosomes following a SNARE mechanism [164].

Several proteins involved in the recognition of LDs for macrolipophagy degradation have been identified (Table 2) but most of them are known as selective autophagy receptors, not specific of lipophagy. This is the case of the adaptor SQSTM1/p62 [165], as well as NBR1 and Optineurin [166]. In addition to these, some lipophagy-specific cargo recognition proteins have been identified in different tissues and conditions. The LD protein ancient ubiquitous protein 1 (AUP1) associates with a E2-ubiquitin ligase and can trigger LDs aggregation [167,168], potentially forming aggresomes to promote lipophagy [166]. Moreover, AUP1 acts as a lipophagy cargo recognition protein following viral infection [169] and has been suggested as a potential target for therapeutic treatment of COVID-19 [170]. ORP8, which is involved in LDs biogenesis at mitochondria-associated ER membrane subdomains [171], can be phosphorylated by AMPK, promoting its role as a lipophagy cargo protein [172]. Finally, two proteins, whose mutants cause hereditary spastic paraplegia diseases, have been shown to act as cargo recognition proteins in lipophagy in the brain: DDH2 [173] and Spartin [174]. The identification of specific lipophagy cargo recognition proteins is of particular interest to understand the regulation of LDs degradation by lipophagy, and in particular the targeting of specific LD populations.

As in lipolysis, PLIN proteins are modified and or degraded to allow lipophagy. PLIN 2 protects LDs from lipophagy [175] and the degradation of PLIN2 and 3 by chaperone mediated autophagy can promote both association of ATGL (as described in section 3) and LC3 proteins at the surface of LDs [60,176]. Moreover, in addition to its above mentioned role, Spartin acts as an adaptor for PLIN2 ubiquitination [177]. PLIN1 colocalizes with p62 [165] and its phosphorylation is necessary to allow Rab7 docking [178], that participates in autophagosome maturation [179], together with other members of the Rab GTPase family like Rab10, 25 and 32 (reviewed in Ref. [146]) (Table 2).

4.2. Microlipophagy in mammals and yeast

The prevalence of macrolipophagy appears specific to mammals as in all other organisms, lipophagy mainly occurs through pathways related to microautophagy.

Microlipophagy was thought to be absent from mammalian cells, but some recent evidence have shown that this pathway plays a significant role in LDs degradation in mammal liver hepatocytes [180]. Indeed, in those cells LDs and lysosomes can form transient contacts that usually last 30–60 s and that do not depend on the canonical macroautophagic machinery pathway, both in presence or absence of nutrients.

In budding yeast, actors and mechanisms involved depend on the condition or stress promoting microlipophagy (Table 2). Microlipophagy was observed in response 1) to nutrients starvation triggered by cell entry into stationary phase or by acute nitrogen or glucose depletion [181–185]; 2) to ER stress mediated by lipid imbalance in a strain with a deficiency in phosphatidylcholine (PC) synthesis, or by the addition of dithiothreitol (DTT) or tunicamycin (TUN), an inhibitor of ER protein glycosylation [186–188] or 3) to diauxic shift triggered by yeast transfer from a fermentative to a respiratory media [189].

Microlipophagy requires the targeting of LDs and attachment to the vacuole membrane, followed by the engulfment of the LDs by an inward invagination of the vacuolar membrane in the lumen (Figs. 2B–1). After scission of the vacuole membrane, the LD is released in the vacuole lumen surrounded by the vacuolar membrane and is then degraded [190,191].

During microlipophagy induced by nutrient starvation, LDs were shown to be recruited at specific microdomains formed in the vacuolar membrane, the liquid-ordered (L_o) microdomains, before their inward budding to promote LD internalization. L_o domain formation is essential for efficient internalization of LDs into vacuoles under nutrient stress and lipid imbalance (Figs. 2B–3) [185,187]. Many proteins required for the formation of these microdomains were identified and their deletion negatively impacts microlipophagy (Table 2) [181,185,192,193]. L_o microdomains are enriched in sterols, contain sphingolipids, that are important for their formation [185,187,193–195] and also PtdIns(3)P and phosphatidylinositol-4-phosphate (PtdIns(4)P) located in the cytosolic leaflet [183,196]. Vacuolar membrane are usually poorly enriched in sterols and sphingolipids implying that these lipids have to be transported to the vacuole membrane when required to form L_o microdomains [194,197]. Several lipid sources and transport mechanisms were proposed: 1) transport from the lumen of sterols coming from the degradation of intraluminal vesicles (ILV) originating from the fusion of sterol-enriched multivesicular bodies (MVB) or from the degradation of LDs after their internalization by two vacuolar sterol transporters, Npc2 and Ncr1 [183,187,198]; 2) transport through non-vesicular pathway, as exemplified by Lam6, a sterol transporter located at the nucleo-vacuolar junction and involved in L_o microdomains formation [192]; 3) transport to the vacuole by autophagy, as autophagosomes that fuse with the vacuole membrane could be a source of sphingolipids and sterols [182,184,185,197]. The involved mechanisms depend on the condition used to induce microlipophagy. Recently, the LD surface proteins Ldo16 and Ldo45, also named LDOs for LD organization, were shown to interact with the vacuolar protein Vac8 and to promote vacuole-LD contact site (vCLIP) formation [199,200]. LDOs are also involved in LD biogenesis as they are associated to the Seipin complex during logarithmic phase [201] and their progressive dephosphorylation when yeasts enter the stationary phase promotes vCLIP formation and lipophagy induction [200]. Therefore, LDOs localization and function could be regulated by phosphorylation dependent on the metabolic status of the cells, connecting LD synthesis to LD degradation.

The mechanisms involved in microlipophagy during ER stress under induction by DTT or TUN present significant differences compared to the ones occurring under nutrient stress. First of all, neither the core autophagy components nor the formation of L_o

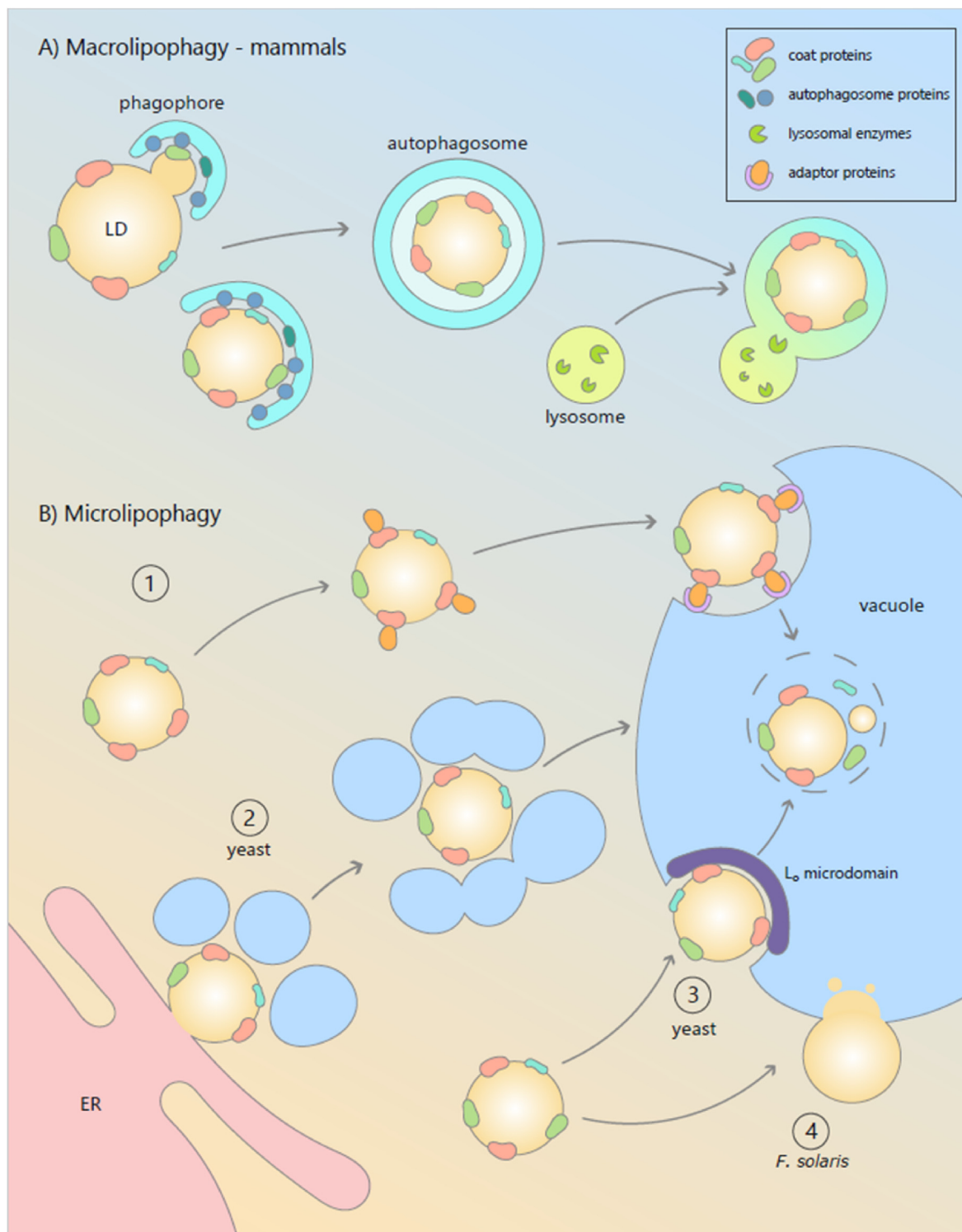


Fig. 2. Overview of lipophagy mechanisms in different organisms. A) Macrolipophagy has been described in mammals: after the formation of the phagophore, the autophagosome completely surrounds the LDs and eventually fuses with lysosomes. B) Microlipophagy has been observed in several eukaryotic organisms, however several mechanisms have been described 1) Specific proteins bind to the LD coat proteins and direct it to the lytic compartment where it is recognized by specific receptors. This broad and simple description matches what has been described in *A. thaliana*, where the coat protein CLO1 appears to regulate addressing to the vacuole, possibly interacting with Atg8, and in yeast, where the LD proteins Ldo16 and Ldo45 can directly bind to the vacuolar protein Vac8 to trigger microlipophagy. 2) In yeast, in response to endoplasmic reticulum (ER) stress, the vacuole fragments into smaller vesicles that organize around the LDs, envelope them and form invagination which release the LDs into the vacuolar lumen following membrane scission; 3) In yeast, during nutrient starvation, LDs can be recruited to the vacuole thanks to specific microdomains (L_0 microdomains) for efficient internalization. 4) In *Fistulifera solaris*, a direct fusion of vacuoles and LDs have been observed without the complete absorption of the LD, suggesting a novel type of microlipophagy.

Table 2

Summary of the proteins shown to play a role in lipid droplet degradation by lipophagy in *Homo sapiens*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*.

	Name	Function	References
<i>S. cerevisiae</i>	Ncr1 and Npc2	Formation and expansion of L _o microdomains in vacuole membrane by transferring sterols from the vacuole lumen to the vacuolar membrane	[183,188]
	Pik4 and Stt4	PtdIns(4) kinases required for local synthesis of PtdIns(4)P into vacuolar L _o microdomains and lipophagy under nitrogen starvation	[196]
	Lam6	Sterol transporter located at nucleo-vacuolar junction and promoting L _o microdomain formation	[192]
	Fab1	Phosphatidylinositides biosynthesis, required for L _o microdomains formation	[185,193]
	Nem	Phospholipid biosynthesis, required for L _o microdomains formation	[185,193]
	Vps4	AAA-ATPase, component of ESCRT complex involved in MVB synthesis, required for L _o microdomains formation	[181,185,188,193]
	Sec18	Involved in vesicular trafficking, required for L _o microdomains formation	[193]
	Pep4	Vacuolar protease, required for L _o microdomains formation	[181,185,188]
	Are1/2	Synthesis of ergosterol esters, required for L _o domain formation	[185]
	ATG15	Vacuolar TAG lipase, degradation of LD TAG in the vacuole after internalization, required for L _o microdomains formation	[166,181]
	Snf1/4	Subunits of the AMP-activated protein kinase complex, activate lipophagy during acute glucose reduction by assisting the relocation of Atg14 to the vacuolar membrane	[182]
	Atg6/14	Part of the phosphatidylinositol 3-kinase complex I that produces PtdIns(3)P. Could play a role in production or targeting of PtdIns(3)P to the vacuole for the formation of L _o microdomains	[181,182,202]
	ESCRT complex	Might assist LDs internalization by promoting vacuole membrane invagination and membrane scission in lipophagy induced by diauxic shift from glycolysis to respiration or ER stress.	[186,188,189]
	Ldo16, Ldo45 and Vac8	Triggers LDs attachment to vacuole via interaction of the LD protein Ldo45 and Ldo16 with the vacuolar membrane protein Vac8	[199,200]
	Core autophagy proteins	Important for LDs internalization into vacuole and for vacuole microdomain maintenance under nutrient starvation	[181–185]
	ATG39/ESM1	Involved in lipophagy under ER-stress triggers by PC imbalance	[188]
	Vam3, Ypt7 and Vps33	Proteins involved in vacuolar membrane fusion and playing a role in lipophagy during ER-stress induced by DTT	[186]
<i>A. thaliana</i>	ATG2/ATG5/ATG7	Involved in microautophagy to promote TAG degradation under starvation induced by extended darkness	[203]
		Regulation of lipid homeostasis in dark-induced senescence	[204]
		TAG degradation under carbon starvation in etiolated seedlings	[205]
	CLO1	LD coat protein interacting with ATG8f to promote LDs internalization into vacuole during lipophagy in germinating seeds	[206]
<i>H. sapiens</i>	SQSTM1/p62, NBR1, optineurin	General selective autophagy receptors, involved in lipophagy	[165,166] +
	Rab7, Rab10, Rab25 and Rab32	LDs targeting to autophagosomes	[146,179]
	Spartin	LDs ubiquitination and recognition for macroautophagy	[174,177]
	AUP1	LDs aggregation and recognition for macrolipophagy following viral infection	[167,169]
	DDH2	LDs recognition for macrolipophagy in the brain	[173]
	ORP8	LDs recognition for macrolipophagy	[172]
	Core autophagy components	Target LDs into vacuole by macrolipophagy	[151]
	LC3	Autophagic cargo selection, phagophore elongation and closure	[207]
	mTOR	Regulator of autophagy, is activated in presence of nutrients	[153]
	PRKA/AMPK	Regulator of autophagy	[146]
	Atg1	Initiator of the phagophore formation	[157]
	LAL/Lipa	Lysozomal lipase involved in LD lipids degradation after internalization by lipophagy	[148–150]
<i>C. reinhardtii</i>	Core autophagy components	Promote TAG accumulation or LDs formation upon nitrogen or phosphate starvation	[208]
	ATG8	LDs degradation by microautophagy during nitrogen resupply	[209]
<i>P. tricornutum</i>	StLDP	Putative interaction with Atg8	[67,108]
	ACBP	Regulation of Atg5?	[210]

microdomains are required [187,188]. In addition, both stresses trigger vacuolar membrane fragmentation; LD internalization is then mediated by the remodeling of membrane vacuoles that organize around LDs, envelop them and form invaginations that release LDs in the vacuolar lumen after membrane scission (Fig. 2B–) [186]. The formation of invaginations and the release of LDs in this situation was proposed to be facilitated respectively by the action of the ESCRT complex and of proteins involved in vacuole fusion rather than by the property of the L_o microdomains as demonstrated under nutrient stress [185–187]. An intermediate situation is observed during lipid imbalance as both the ESCRT complex and the L_o microdomains formation mediated by Npc2 and Ncr1 are required [187], demonstrating the co-existence of different pathways mediating LD microlipophagy.

Once in the vacuoles, degradation of LD proteins and lipids could

involve the Pep4 protease and the Atg15 lipase, that are located in the vacuole lumen and play a role in microlipophagy under both nutrient starvation and ER stress [166,182,188]. More recently, as for PLIN2 and PLIN3 in mammals (see section 3), autophagy was shown to be required for the removal of LD surface proteins prior to their internalization into vacuole under nutrient stress [181], shedding light on the poorly understood role of general autophagy in microautophagy. However, protein removal is not a pre-requisite for LD entry in the vacuole suggesting that LD protein removal and LD entry in the vacuole for lipolysis are two independent processes [181].

4.3. Microlipophagy-like processes in plants and algae

In plants, the number of studies investigating the involvement

of autophagy core components in LD degradation is limited and results are variable depending on the species, the developmental stage, the tissue and/or the stress analyzed. During seed germination, TAG are mainly degraded by the cytosolic TAG lipases SDP1 and SDPL1 that are essential for seed germination in absence of carbon source in the media (see section 2). However, degradation of LDs by a process similar to microlipophagy was also observed in *A. thaliana* where a subset of LDs were located in the vacuoles 36–48h after germination [206,211]. In addition, autophagy inhibition by concanamycin A negatively impacts seedling growth after germination and leads to a high accumulation of LDs in cotyledons, likely due to a lower level of degradation compared to WT seedlings [206]. LDs accumulation into vacuoles was also observed in other plant tissues including in tapetum cells during pollen development in rice as well as under dark-induced starvation in *A. thaliana* leaves [203,212], suggesting that microlipophagy could be an important process involved in plant development and stress response. Microlipophagy in plants involves core components of the autophagy machinery as the absence of ATG7 in rice, or ATG2 and ATG5 in *A. thaliana* significantly reduces the accumulation of LDs into vacuoles [203,212]. In addition, ATG8e and b, key autophagy proteins involved in cargo selection and autophagosome biogenesis, were co-localized with LDs in leaves under dark stress and seedlings during germination, respectively [203,206]. During germination, the seed specific LD coat protein of the caleosin family CLO1 is also a key player in microlipophagy as CLO1 seems to regulate the entry of LDs into the vacuole during germination, but the exact mechanism remains to be investigated [206,211]. CLO1 could regulate LD-vacuole interactions and/or promote membrane fusion. In other plant tissues or stress conditions, mutations of autophagy core components can lead to a reduction of TAG and/or LD degradation. This is the case in tobacco pollen and maize leaves, as well as in *A. thaliana* seedlings under carbon starvation or leaves under dark-induced senescence [203,205,213,214]. However, the involvement of lipophagy in plants is not limited to lipids degradation but seems to play a more central role in lipid metabolism and homeostasis. Indeed, in leaves upon dark-induced senescence, ATG proteins were also shown to be involved in TAG synthesis and the authors propose that autophagy promotes TAG synthesis from membrane lipid turnover in specific tissues or developmental stages, while it is involved in TAG degradation in other tissues, such as pollen or seedlings, or under different stresses [203,204]. A similar view has been proposed in the green alga *Chlamydomonas reinhardtii*, where inhibition of the autophagic flux prevents TAG accumulation and LD formation upon nitrogen or phosphate starvation [208].

Studies of lipophagy in microalgae are only emerging, but more and more evidence of a role of this process in LD degradation are reported in the literature. Inclusion of LDs in vacuoles were initially observed in the green alga *Auxenochlorella protothecoides*, during heterotrophy to autotrophy transition [215]. Similarly, LDs are found in the vacuolar lumen of *Parachlorella kessleri* following salt stress [216] and in *C. reinhardtii*, micropagy-like interactions of LDs and vacuoles were observed during nitrogen resupply following starvation [217]. Like in plants, ATG8 is involved in lipophagy processes in *C. reinhardtii*. Indeed, LDs directly interact with ATG8-labeled structure under nitrogen starvation [218] and TAG degradation during nitrogen resupply is delayed in CrATG8 mutants [209,219]. While most lipophagy mechanisms in green microalgae are described as “micropagy-like”, the involvement of ATG8, a protein involved in autophagosome formation, and the description of autophagosome-like vesicles in both *C. reinhardtii* [218] and *Micrasterias denticulata* [220] suggest that macroautophagy processes can also be involved. The articulation between micro- and macroautophagy processes remains unclear.

Micropagy-like degradation of LDs and involvement of ATG8 are also observed in Stramenopiles. In *Nannochloropsis oceanica*, micropagy-like degradation of LDs during nitrogen resupply and interactions between NoATG8 and NoLSDP, the major LD protein in *N. oceanica*, have been observed [221]. An ATG8-interacting motif has also been identified in the Stramenopile lipid droplet protein (StLDP), the major protein at the surface of LDs in the diatom *P. tricornutum* [108,110], whose mutants show an overaccumulation of TAG and impaired LD degradation following nitrogen replenishment [67]. Similarly, a recent study has highlighted the importance of an acyl-binding protein (ACBP) in LD degradation and showed that impaired LD degradation in the ACBP KO could be linked to autophagy defects as *atg5* expression was repressed in these mutants [210]. Yet, the exact role of ACBP remains elusive and the role of lipophagy in LD degradation in *P. tricornutum* remains hypothetical. However, lipophagy has been shown to play a role in lipid degradation in another diatom, *Fistulifera solaris* [222]. Electron microscopy images suggested that in *F. solaris*, LD monolayer could directly fuse with the vacuole membrane, leading to the delivery of their lipid content directly in the vacuole lumen without complete LD internalization (Figs. 2B–4), suggesting that a microlipophagy pathway, different from what is described in yeast, could also exist in algae [222].

5. Conclusion

The degradation of LDs follows similar general pathways in all organisms considered in this review, and some key core components, involved in the initial steps of LD degradation, appear conserved. This is the case for the main TAG lipases, that orchestrate hydrolysis of TAG to DAG (Table 1) and for the general autophagy machinery. However, the following steps show a great diversity, depending on organisms but also tissue/organ within the same organism, and subsequent fate of released fatty acids. CGI-58 is an interesting example in the lipolysis process. Indeed, while the protein is conserved at the sequence level and has been identified in all considered phyla, its localization and function vary (cf. section 3.3). In plants, CGI-58 is localized at the surface at peroxisomes and interacts with PXA1 [73], likely to regulate FA entry into β -oxidation following TAG degradation (see Fig. 1) and the subsequent use of these FA as hormone precursors of the jasmonate and auxin biosynthetic pathways, particularly in vegetative tissues [73]. By contrast, localization at the surface of LD in mammals allows a wider range of fates of released FA [101], and association of CGI-58 with mitochondria in microalgae can serve to balance the fate of FA between degradation and recycling [77].

Lipophagy, and in particular microlipophagy, shows an even broader diversity of routes and players (Fig. 2 and Table 2), suggesting that it has evolved differently in the considered phyla. However, it is important to note that knowledge regarding lipophagy is only recent and partial, as illustrated by the recent discovery of microlipophagy in human [180]. Our vision may thus be biased by research focus that differs between phyla. Indeed, in mammals, defects in LD formation or degradation are associated with many diseases, including metabolic diseases but also central nervous system diseases (for review [223]). Metabolic diseases and the associated tissues (adipose tissue and liver) have consequently been a major focus of LD research. In plants, studies have for a long time focused on oleaginous seeds and germination, leaving aside other tissues or processes where LDs seem much less prominent but play essential roles. Finally, in microalgae, a lot of research is turned towards the increase or alteration of TAG content for biotechnological applications. It is therefore possible that the differences in lipophagy, and more generally LD degradation pathways relate to the physiology and/or functions of studied LDs in the

considered tissue/condition of one organism. An interesting example of lipophagy-specific function has been shown in response to ER stress in yeast, where microlipophagy was suggested to contribute to ER quality control promoting cell survival through the removal of damaged proteins from the ER, as well as through the removal of lipid excess observed during lipid imbalance [186,188]. Indeed, several cargo proteins, some being polyubiquitinated, are associated to LDs under stress and are internalized into vacuole with LDs for degradation, demonstrating the role of microlipophagy in proteostasis under ER stress [186,188]. Investigating similar mechanisms in other organisms would be very interesting, especially in other unicellular organism as some very interesting common points can be drawn. We have highlighted in part 3.2.1 the relationship between cell cycle and LD degradation in unicellular organisms, that relates to their life cycle. Another very interesting feature is the close link between LD biogenesis and degradation that seems to exist within these organisms. Indeed, while in mammals and plants, described functions of LD coat proteins are related to protection against LD degradation (cf. part 3), the major LD proteins of two distant stramenopiles, *N. oceanica* and *P. tricornutum*, are involved in LD degradation, probably through lipophagy (cf. part 4, [67,221]). In yeast, the LDO proteins are both involved in the Seipin complex and in LD degradation through microlipophagy (cf. part 4, [199,200]). Thus, in these organisms, that need to quickly respond to environmental changes, LDs seem to be primed for degradation during their biogenesis. This could also be the case in some tissues of pluricellular organisms. In plant leaves, the LD coat protein LDIP also interacts with Seipin and seems to be functionally homologous to LDO45 [224], while mammalian ORP8 seems involved in both LD biogenesis and lipophagy [171,172](cf. section 4.1).

Finally, in the present work we have presented lipolysis and lipophagy separately, but the degradation of large LDs highlights the integration of both main routes, and its possible conservation. In the green microalga *Auxenochlorella protothecoides*, it has been suggested that large LDs are first broken down by lipolysis to reduce their size, which allows their subsequent degradation by lipophagy [215]. Observations of LDs dynamics following nitrogen replenishment in *P. tricornutum* suggest that a similar mechanism could be at play, as two phases are observed: a first one during which the number of LDs increases while their size decreases, and a second where the number and size of LDs decrease [225]. The existence of non-essential lipophagy to degrade LDs in plant seeds [206,211] and adipocytes [178], as well as the involvement of ATGL [226,227] and other patatin-like lipases (reviewed in Ref. [228]) in lipophagy in the liver and brown adipose tissue, suggest that similar cooperation of lipolysis and lipophagy could facilitate the degradation of large LDs in these tissues.

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CRediT authorship contribution statement

Chems Amari: Writing – review & editing, Writing – original draft, Conceptualization. **Marta Carletti:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Siqi Yan:** Writing – review & editing, Writing – original draft, Conceptualization. **Morgane Michaud:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Juliette Salvaing:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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