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Chapter 11

The Puzzling Conservation and Diversification of Lipid Droplets from Bacteria to Eukaryotes

Josselin Lupette and Eric Maréchal

Abstract Membrane compartments are amongst the most fascinating markers of cell evolution from prokaryotes to eukaryotes, some being conserved and the others having emerged via a series of primary and secondary endosymbiosis events. Membrane compartments comprise the system limiting cells (one or two membranes in bacteria, a unique plasma membrane in eukaryotes) and a variety of internal vesicular, subspherical, tubular, or reticulated organelles. In eukaryotes, the internal membranes comprise on the one hand the general endomembrane system, a dynamic network including organelles like the endoplasmic reticulum, the Golgi apparatus, the nuclear envelope, etc. and also the plasma membrane, which are linked *via* direct lateral connectivity (e.g. between the endoplasmic reticulum and the nuclear outer envelope membrane) or indirectly *via* vesicular trafficking. On the other hand, semi-autonomous organelles, i.e. mitochondria and chloroplasts, are disconnected from the endomembrane system and request vertical transmission following cell division. Membranes are organized as lipid bilayers in which proteins are embedded. The budding of some of these membranes, leading to the formation of the so-called lipid droplets (LDs) loaded with hydrophobic molecules, most notably triacylglycerol, is conserved in all clades. The evolution of eukaryotes is marked by the acquisition of mitochondria and simple plastids from Gram-positive bacteria by primary endosymbiosis events and the emergence of extremely complex plastids, collectively called secondary plastids, bounded by three to four membranes, following multiple and independent secondary endosymbiosis events. There is currently no consensus view of the evolution of LDs in the Tree of Life. Some features are conserved; others show

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a striking level of diversification. Here, we summarize the current knowledge on the architecture, dynamics, and multitude of functions of the lipid droplets in prokaryotes and in eukaryotes deriving from primary and secondary endosymbiosis events.

Keywords Lipid droplets · Evolution · Architecture · Biogenesis · Catabolism

11.1 Introduction

AU2

Lipid droplets (LDs) are conserved structures in prokaryotic and eukaryotic cells (Walther et al. 2017; Zhang and Liu 2017). Their architecture consists of a core, loaded with hydrophobic carbon-rich molecules (polyhydroxyalkanoates or PHAs, triacylglycerol or TAG, sterol esters, isoprenoids such as squalene, etc.) bounded by a monolayer of polar glycerolipids, generally phospholipids that can be associated with sterols. Proteins are transiently or permanently associated with its surface (Walther et al. 2017) (Fig. 11.1). Two main classes have been identified amongst LD closely associated proteins, based on their structure (Kory et al. 2016). Class I proteins have a hydrophobic “hairpin” pattern (Bersuker and Olzmann 2017) and class II proteins have at least one amphipathic helix (Bersuker and Olzmann 2017) (Fig. 11.1). Nevertheless, proteins that do not belong to these two classes are also encountered.

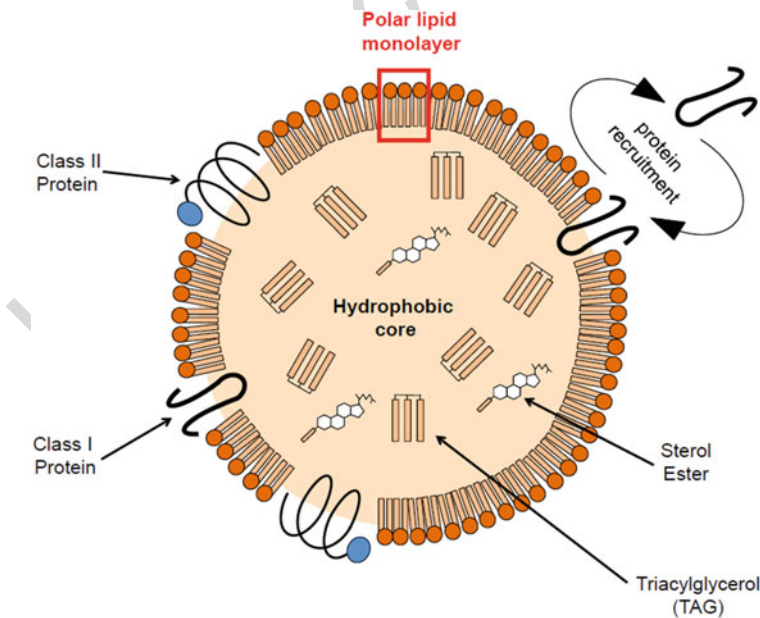


Fig. 11.1 General architecture of a triacylglycerol-containing lipid droplet

LDs derive from the budding of a cell membrane. Membrane compartments are amongst the most fascinating markers of cell evolution from prokaryotes to eukaryotes, some being conserved and the others having emerged via a series of primary and secondary endosymbiosis events. Membrane compartments comprise the system limiting cells (one or two membranes in bacteria, a unique plasma membrane in eukaryotes) and a variety of internal vesicular, subspherical, tubular, or reticulated organelles. In eukaryotes, the internal membranes comprise on the one hand the general endomembrane system, a dynamic network including organelles like the endoplasmic reticulum (ER), the Golgi apparatus, nuclear envelope, etc. and also the plasma membrane, which are linked *via* direct lateral connectivity (e.g. between the endoplasmic reticulum and the nuclear outer envelope membrane) or indirectly *via* vesicular trafficking. On the other hand, semi-autonomous organelles, i.e. mitochondria and chloroplasts, are disconnected from the endomembrane system and request vertical transmission following cell division. Membranes are organized as lipid bilayers in which proteins are embedded. The evolution of eukaryotes is marked by the acquisition of mitochondria and simple plastids from Gram-negative bacteria by primary endosymbiosis events (Maréchal 2018), and the emergence of extremely complex plastids, collectively called secondary plastids, bounded by three to four membranes, following multiple and independent secondary endosymbiosis events (Fussy 2018). There is currently no consensus view on the evolution of cell membranes and that of LDs.

Due to their hydrophobic core loaded with carbon-rich molecules, LDs have long been considered as simple carbon and energy storage organelles. Based on the analyses of LD proteomes in various cell models, it is now considered that LDs have other functions that depend on their protein composition (Walther et al. 2017; Den Brok et al. 2018; Henne et al. 2018).

Research on LDs has increased strikingly in recent decades motivated by the multitude of applications ranging from nutrition, health to green chemistry and bioenergy. In 2020, the keyword “lipid droplet” returns as many as 12,500 hits in the Pubmed bibliographic database. Concerning human obesity-related diseases (Faucher and Poitou 2016; Madrigal-Matute and Cuervo 2016), protein actors (CGI-58, SEIPIN, ATGL, LAL) at the surface of LDs have been extensively studied in mammalian models (summarized in Table 11.1). CGI-58-ABHD5 (Comparative Gene Identification 58— α/β hydrolase domain-containing 5; 349 amino acids—45 kDa) is particularly studied because its mutation is responsible for the Chanarin-Dorfman syndrome, an autosomal recessive disease (Missaglia et al. 2014). In mammals, the CGI-58 protein is located on cytosolic LDs interacting with PLIN1 (Subramanian et al. 2004). A second important player in the mechanisms of LD biogenesis in mammals is the SEIPIN protein. A mutation in the human Seipin gene leads to severe forms of generalized Berardinelli-Seip congenital lipodystrophy (Magré et al. 2001). The deletion of ATGL (Adipose triglyceride lipase) in mice reduces the mechanism of lipolysis and promotes the accumulation of lipids in oxidative tissues of the body, leading to the death of mice in 3 months (Zimmermann et al. 2004). The last example is the Wolman disease, which is an autosomal recessive disease affecting young children following a mutation in the

Table 11.1 Human diseases related to LD formation

	Diseases	Anatomical pathology	Pathophysiology	References
t1.3	Atherosclerosis	Accumulation of atheromatous plaques (cholesterol) in the arteries	ACAT1; ABCA1; ADRP	Paul et al. (2008)
t1.4	Obesity	Accumulation of fat reserves	Multifactorial (genetic, environmental, psychological)	Faucher and Poitou (2016)
t1.5	Fatty liver	Accumulation of TAGs in the cytoplasm of hepatocytes	Alcohol, hepatitis B and C	Madrigal-Matute and Cuervo (2016)
t1.6	Chanarin-Dorfman syndrome	Accumulation of lipid droplets in lymphocytes and many tissues	Mutation of CGI-58/ABHD5	Dorfman et al. (1974), Chanarin et al. (1975), Lefèvre et al. (2001), Samuelov et al. (2011), Missaglia et al. (2014), Jordans (1953), Gupta and Kaur (2005), Waheed et al. (2016)
t1.7	Myopathy		Mutation of PNPLA2	Zimmermann et al. (2004)
t1.8	Congenital generalized Lipodystrophy (CGL)	Dystrophy of adipose tissue	Mutation of AGPAT2, BSCL2, CAV1 or PTRF	Magré et al. (2001), Agarwal et al. (2002), Kim et al. (2008), Hayashi et al. (2009), Rajab et al. (2010), Quinn and Purcell (2017)
t1.9	Lysosomal acid lipase deficiency	Lysosomal acid lipase deficiency causing an accumulation of TAGs and cholesterol esters in leukocytes, hepatocytes, and fibroblasts	Mutation of LIPA	Wolman et al. (1961), Onal et al. (2017), Pericleous et al. (2017), Ikari et al. (2018)

LIPA gene (Wolman et al. 1961). The LIPA mutation leads to the synthesis deficiency of lysosomal acid lipase (LAL) (Onal et al. 2017). This disease causes accumulation of cholesterol esters and TAG in leukocyte lysosomes, fibroblasts, and hepatocytes generally leading to the death of the child by liver failure (Pericleous et al. 2017). It is a very rare disease with only 14 cases detected so far, half of which are from the consanguineous union (Ikari et al. 2018).

TAG-rich LDs produced by oleaginous organisms, mainly plants and algae, but sometimes also fungi or animals, are also key to numerous biotechnological applications. Molecules of TAG are composed of a glycerol-3-phosphate backbone on which three fatty acids are esterified (Lupette and Maréchal 2018). Fatty acids (FAs) are carboxylic acids. Their carbon chain length and number of unsaturations (or double bonds, C=C) allow assessing whether they can be used for different applications. Oleaginous crops are an essential resource for human nutrition. Microalgae, whose interest in the scientific community is currently exponential,

can also produce TAGs. Microalgae enriched in FAs with short or medium carbon chains without unsaturation are an interesting feedstock for green chemistry or the development of biofuels (Lupette and Maréchal 2018). Microalgae containing high levels of very long-chain fatty acids (carbon number greater than 20) with multiple unsaturations (1–6), called VLC-PUFAs, with unsaturation at the ω -3 position (i.e. eicosapentaenoic acid or EPA, 20:5, and docosahexaenoic acid or DHA, 22:6), are promising for human health applications (Lupette and Maréchal 2018).

There is currently no consensus view of the evolution of LDs in the Tree of Life. Some features are conserved; others show a striking level of diversification. Here, we summarize the current knowledge on the architecture, dynamics, and multitude of functions of the lipid droplets in prokaryotes and eukaryotes deriving from primary and secondary endosymbiosis events.

11.2 Studying Lipid Droplets

11.2.1 Imaging Lipid Droplets

Microscopic observation by confocal or epifluorescence imaging is the main method of detection of LDs in a cell or an organelle. The most commonly used fluorophores are Nile Red (Greenspan et al. 1985) and BODIPY 505/515 (Rumin et al. 2015) or BODIPY 493/503 (Gocze and Freeman 1994). More recently, new fluorophores have been developed (Yang et al. 2012; Gidda et al. 2016). These molecules are compatible with the parallel measurement of the fluorescence of GFP (Green Fluorescent Protein), RFP (Red Fluorescent Protein), and of chlorophyll (Kuntam et al. 2015). Other compounds including AC-202 were recently used in two model species of microalgae *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* (Harchouni et al. 2018). These fluorophores make it possible to determine the size and number of LDs in a semi-quantitative manner, as well as their cellular localization.

11.2.2 Purifying Lipid Droplets

The general strategy for studying LD architecture is similar regardless of the organism studied. A culture of cells in a medium promoting the development of LDs is used (e.g. a nutrient deficiency). The LD purification starts by a gentle cell disruption step (French press, cell disruptor, etc.) in a suitable buffer releasing droplets as well as other cellular components. It is then necessary to perform a density gradient purification (Brasaemle and Wolins 2016). Due to their low density, LDs rise to the surface of the gradient during ultracentrifugation (Brasaemle and Wolins 2016). LDs are harvested and washed to limit the presence of contaminants. The study of LD architecture is based first on the determination of all the

components of the hydrophobic core and on the composition of the monolayer of polar lipids and proteins on the surface of the droplet (Walther et al. 2017). Proteomic analyses allow the identification of proteins but also the characterization of some post-translational modifications (phosphorylation, nitrosylation, ubiquitinylation, sumoylation, *N* and *O*-glycosylation, farnesylation). The proteome must then be validated by biochemical studies (western blot), the imaging of fusion proteins (with a fluorescent marker to verify the location on the surface of LDs), and functional genetic studies (the study of mutants with altered expression such as knockout, silencing, and overexpression of genes coding for droplet proteins allows their functional characterization).

11.2.3 Biophysical Properties of Lipid Droplets

Biophysical studies of LDs have proven to be critical to advance our understanding of LD biogenesis. After the removal of proteins and polar lipids, it is possible to consider LDs as the product of an emulsion of oil in water (Thiam et al. 2013b). The cytosol of the cell represents the aqueous phase and LDs, the dispersed oily phase. The interface between oil and water generates a surface tension due to the lack of cohesive integrations between the two phases. These emulsions are metastable in the absence of external disturbance. The presence of surfactants makes it possible to reduce the surface tension, thus increasing the (meta)stability of the emulsion and the cohesion energy cost (Georgieva et al. 2009). The polar glycerolipids at the periphery of the droplet then act as surfactants. Mastering this system in vitro is probably one of the most important challenges to understand how such anisotropic hydrophobic cores can be maintained in a cell, where all other components are highly self-assembled and organized (membranes can be considered as two-dimensional fluid crystals, DNA and polymers have three-dimensional architectures, polypeptides form protein structures with rigorous three-dimensional folds). It is also possible to use biophysical methods such as Pulsed Field Gradient-Nuclear Magnetic Resonance (PFG-NMR) to determine the mobility of TAGs inside the volume set by LDs with or without LD-to-LD connections (Gromova et al. 2015). Since the overall structure of LDs appears conserved in the Tree of Life, whereas components may differ, a key to LD conservation may rely on biophysical properties, which now need to be evaluated in different systems.

11.3 Lipid Droplets in the Tree of Life

11.3.1 Lipid Droplets in Prokaryotic Cells

The vast majority of bacterial species have the capacity to accumulate lipid compounds within their cytoplasm, especially during nutritional stress. A distinction is

made according to accumulated hydrophobic molecules, with either “lipid droplets” (containing fluid acyl esters, triacylglycerols, or TAGs) or “granules” (containing semi-solid lipopolymers called polyhydroxyalkanoates or PHAs). Here, we describe the formation of LD structures in bacteria, as a possible basis for their evolution in eukaryotes following primary endosymbiosis events. Since primary endosymbiosis events are believed to be facilitated by the presence of pathogenic partners (Maréchal 2018), we also describe how some pathogenic bacteria (and viruses) are known to interact with host cell LDs.

11.3.1.1 Polyhydroxyalkanoate Granules

Polyhydroxyalkanoates (PHAs) are polyesters produced by fermentation of lipids or carbohydrates. They are linear polyesters consisting of hydroxy acid monomers (HA) linked together by an ester bond (Możejko-Ciesielska and Kiewisz 2016). PHAs include poly(3-hydroxybutyrate) (PHB) and polyhydroxyvalerate (PHV) (Murphy 2012). PHAs are also classified into two groups according to the number of carbons per monomer: short-chain (3–5 carbons) PHAs and medium-chain PHAs (6–14 carbons). PHAs are synthesized when the C/N ratio is altered (a nitrogen deficiency coupled with an excess of carbon), stopping growth and division, resulting in an entry into the quiescent phase. Special attention has been given to these polyesters for several decades because they are biodegradable (Pötter and Steinbüchel 2006). PHAs are used in a wide range of applications such as resorbable materials for medical purposes (implants, biodegradable sutures, stents, etc.), materials (paper coating, shape memory gel, etc.), fuel additives, and as metabolic regulators (Możejko-Ciesielska and Kiewisz 2016).

Ralstonia eutropha H16 (new name: *Cupriavidus necator*) is the study model for PHB granules (Reinecke and Steinbüchel 2009). It is a Gram-negative bacterium that can accumulate 10–20 granules per cell, measuring 500 nm in diameter and representing 90% of the dry weight (Anderson and Dawes 1990). A recent study has shown that *R. eutropha* H16 PHB granules do not have a monolayer of polar lipids but only superficial proteins essential for their synthesis and degradation (Bresan et al. 2016). The main proteins detected on their surface are PHB synthase (PhaC), phasin (PhaP), PhaR (PhaR), and PHB depolymerase (PhaZ). The *R. eutropha* H16 PhaC gene has been cloned by three independent laboratories. The localization of the PHB synthase on the surface of the granules was confirmed by immunocytochemical staining with colloidal gold (Gerngross et al. 1993). The study of PHA granules is still too scarce to assess whether they are linked to TAG containing LDs and whether they may also be present in some eukaryotic clades.

11.3.1.2 TAG and Wax Ester Droplets

Bacteria are also able to accumulate TAG in LDs. Bacterial species producing LDs have been described in the following genera: *Mycobacterium*, *Nocardia*,

215 *Rhodococcus*, *Micromonospora*, *Dietzia*, and *Gordonia* as well as several Strepto-
 216 mycetes (Alvarez and Steinbüchel 2002; Wältermann and Steinbüchel 2005; Mur-
 217 phy 2012). γ -Proteobacteria (*Marinobacter*, *Alcanivorax*, etc.) are hydrocarbon-
 218 based bacteria capable of accumulating LDs (TAGs and wax esters) when entering
 219 dormancy (Kalscheuer et al. 2007). These γ -proteobacteria are often found in the
 220 oceans associated with microalgae in a system called the phycosphere (Lupette et al.
 221 2016). These bacteria are also able to use petroleum hydrocarbons as a source of
 222 carbon raising possible applications in the degradation of hydrocarbons during oil
 223 spills (Murphy 2012).

224 *Rhodococcus* are oleaginous bacteria containing large amounts of TAGs (Alvarez
 225 2016). Proteomic studies of LDs of several *Rhodococcus* species have been
 226 performed: *Rhodococcus opacus* and *Rhodococcus ruber* (Kalscheuer et al. 2001),
 227 *Rhodococcus jostii* RHA1 (Ding et al. 2012b), and *Rhodococcus opacus* PD630
 228 (Kalscheuer et al. 2001; Chen et al. 2014). 228 proteins have been identified in
 229 *R. jostii* RHA1 including two putative structural proteins representing 15% of LD
 230 proteins: Microorganism Lipid Droplet Small (MLDS) and Phage shock protein A
 231 (Psp A). Ribosomes and translational regulators have also been isolated in the
 232 proteome of the LD of *R. jostii* RHA1 (Ding et al. 2012b). By a functional genetic
 233 study, deletion of the MLDS causes the formation of larger LDs (Ding et al. 2012b).
 234 A recent study showed that LDs of *R. jostii* RHA1 are bound to genomic DNA via
 235 MLDS protein, which increases the survival rate of bacterial cells during nutritional
 236 deficiency or genotoxic stress (Zhang et al. 2017).

237 11.3.1.3 Pathogenic Bacteria

238 Pathogenic bacteria can divert lipids and even ‘hijack’ LDs from an infected host.
 239 Well-known examples include bacteria of the genera *Mycobacterium* and *Chla-*
 240 *mydia*. Mycobacteria are bacilli with pathogenic potency: *Mycobacterium tubercu-*
 241 *losis* (Menon et al. 2019), *M. bovis*, and *M. avium* are causative agents of
 242 tuberculosis, *M. leprae* is the agent of leprosy, and *M. ulcerans* is responsible for
 243 Buruli ulcer. *Mycobacteria* are able to disrupt human lipid homeostasis during
 244 infection following the formation of foamy macrophages containing LDs in the
 245 cytoplasm (Kim et al. 2010; Caire-Brändli et al. 2014). LDs produced in infected
 246 cells serve as a platform for the production of signaling molecules (prostaglandins
 247 and leukotrienes eicosanoids) regulating the immune response and inflammation
 248 (Melo and Weller 2016). LDs also serve to concentrate and deliver iron via lipophilic
 249 siderophores (mycobactins) secreted by the bacteria (Luo et al. 2005). The LD
 250 proteome of *Bacillus Calmette and Guérin* (BCG: a non-replicating hypoxic atten-
 251 uated strain of *M. bovis*) used in the tuberculosis vaccine allowed the identification
 252 of five proteins: two triacylglycerol synthases, Tgs1 (BCG3153c) and Tgs2
 253 (BCG3794c), as well as three other proteins, BCG1169c, BCG1489c, and
 254 BCG1721 (Low et al. 2010). BCG1169c is a specific protein of the *Mycobacterium*
 255 clade. BCG1489c codes for a putative AGPAT involved in the formation of PA in

the Kennedy pathway. A deletion of BCG1489C causes a decrease in the amount of TAG.

Chlamydia trachomatis is an obligate intracellular bacterium responsible for sexually transmitted infections as well as eye diseases. This bacterium has a biphasic life cycle: the elementary body representing the infectious form and the non-infectious reticulate body. *C. trachomatis* is able to translocate an LD from the cytoplasm of the host to the parasitophorous vacuole lumen containing the bacterium via an endocytosis process. Internalization occurs after LDs are coated with a family of Lda proteins (Kumar et al. 2006). The proteome of LDs of HeLa human cervical adenocarcinoma epithelial cells infected with *C. trachomatis* LGV-L2 434/Bu showed that they are enriched in PLIN2, PLIN3, ACSL-3, and ACSL-4 proteins (Saka et al. 2015).

11.3.2 Hijacking of Lipid Droplets by Viruses

LDs can serve as a source of energy for the dispersion of viruses such as hepatitis C virus (HCV), dengue virus (DENV), poliovirus (PV), or rotavirus (RV). This section presents several examples of ‘hijacking’ of lipid metabolism by viruses.

HCV is the most widely used model for studying LD diversion (Roingeard and Melo 2017). The virion initially circulates in a form of lipoviroparticles (rich in cholesterol esters and apolipoproteins apoB and apoE) in the blood of infected patients (Boyer et al. 2014). Its entry into the cell is dependent on LDL receptors (Low Density Lipoproteins). The replication of the viral RNA then induces extensive alterations of the membrane and the formation of vesicular structures that exhibit features similar to lipid rafts (Aizaki et al. 2004). The assembly of HCV requires the localization of some of the HCV proteins in LDs of the host, and the release of virions is strongly associated with the secretion pathway of very low-density lipoproteins (VLDL). Proteins that are important to complete the viral cycle, i.e. the nucleocapsid of HCV and the nonstructural protein NS5A co-localize with the LD in HCV-infected cells and interact with LD proteins, such as DGAT1 (Camus et al. 2013).

Other examples of viruses depending on host cell LDs to complete their cycle include DENV in mosquitoes (Mayer et al. 2017), PV causing poliomyelitis (Nchoutmboube et al. 2013), Flock House Virus (Castorena et al. 2010), Brome mosaic virus (BMV) (Zhang et al. 2016), or RV causing gastroenteritis in infants and young children (Gaunt et al. 2013).

11.3.3 Lipid Droplets in Eukaryotic Cells

Eukaryotes including unicellular and multicellular organisms are characterized by the presence of membrane organelles (endoplasmic reticulum, nucleus, Golgi,

trans-Golgi network, peroxisomes, lysosomes, vacuoles, and numerous cytosolic vesicles for the endomembrane system, mitochondria, and chloroplast for semi-autonomous organelles). Primary endosymbiosis events are at the origin of mitochondria and primary plastids. In a very simplistic scheme, mitochondria are considered to derive from Gram-negative alpha-proteobacteria and primary plastids, from Gram-negative cyanobacteria. Based on molecular evidence, other partners have been involved, including pathogenic bacteria (Maréchal 2018). In contrast to prokaryotes, the synthesis of LDs from the ER seems to occur nearly in all eukaryotes studied to date.

In this section, we first consider non-photosynthetic eukaryotes, containing mitochondria as unique semi-autonomous organelles, then photosynthetic eukaryotes containing primary plastids, and finally those containing secondary plastids.

11.3.3.1 *Opisthokonta: Non-photosynthetic Eukaryotes*

Metazoa

Chordata

LDs of mammals are composed of a hydrophobic core consisting of TAGs and cholesteryl esters, generally considered as a form of storage. The monolayer of polar lipids is mainly composed of PC, PE, and PI (Bartz et al. 2007). In mammals, LD surface proteins are grouped into a family formerly called PAT (for Perilipin—Adipophilin—Tail-interacting protein of 47 kDa) (Bickel et al. 2009) but whose nomenclature has evolved since 2010 under the name of Perilipin (PLIN) (Kimmel et al. 2010). PLIN1 has four splice variants PLIN1a, PLIN1b, PLIN1c, and PLIN1d (Kimmel et al. 2010). PLINs contain a hydrophobic PAT domain of 100 amino acids defining an N-terminal region. The study of a mutant PLIN2-N1 (deficient for the PAT domain) reveals that this domain is not involved in lipid binding, but in the stabilization of lipid droplets, and lipid accumulation and degradation of PLIN2 by the proteasome (Najt et al. 2014). The PAT domain is followed by an 11-mer helical pattern of variable size, which might interact with phospholipids (Bussell and Eliezer 2003). Some PLIN proteins can be post-translationally modified by phosphorylations, via PKA, during lipolysis (PLIN3 and PLIN4 do not have a phosphorylation site (Kimmel and Sztalryd 2016)). Recently, the sixth clade of Perilipin, called PLIN6, has been discovered, specific to teleosts (Granneman et al. 2017). Interestingly, PLIN6 is not expressed in tissues associated with lipid metabolism but in the xanthophores of teleost skin. Biochemical analyses have shown that PLIN6 is associated with the surface of droplets enriched in carotenoids and regulates the pigment synthesis pathways (Granneman et al. 2017).

The expression of PLINs (with the exception of PLIN3) is regulated by a family of transcription factors called PPAR (Peroxisome Proliferator Activated Receptor). These transcription factors are activated by the binding of lipid ligands (Poulsen et al. 2012).

Since the early 2000s, at least 25 proteomic studies of the LD of mammalian cells or tissues have been published (Table 11.2). In all these studies, proteomics reveals the presence of at least one of the classes of PLIN on the surface of the mammalian LD. It can also be seen that the distribution of the PLINs varies according to mammalian cells and tissues. PLIN1 and PLIN4 are present on the surface of LDs of adipocytes (Ding et al. 2012a) and adipose tissue (Yu et al. 2015). PLIN2 and PLIN3 are ubiquitous in non-adipose tissues. PLIN2 is strongly expressed on the surface of LDs of the liver and hepatocytes. PLIN5 is present in the oxidative tissues i.e. the heart, brown adipose tissue, and skeletal muscles (Kimmel and Sztalryd 2014).

Functional studies of PLIN have been performed upstream and downstream of proteomic studies. KO mice for PLIN1 have a phenotype of reduction of fat mass, an increase of the lipolytic activity, but also a glucose intolerance and peripheral insulin resistance (Tansey et al. 2001). Stimulation of lipolytic activity by tumor necrosis factor (TNF- α) showed, in a first study, a decrease in the expression of PLIN1 variants. However, the overexpression of PLIN1a and PLIN1b blocks the ability of TNF- α to increase lipolysis in 3T3-L1 cells (Souza et al. 1998). KO mice for PLIN2 present unchanged adipose differentiation, a 60% decrease in hepatic TAGs, but a level of VLDL identical to control mice suggesting the retention of TAGs in the microsomes (Chang et al. 2006). Overexpression of PLIN2 fused to GFP causes an increase in the number and size of LDs in hepatocytes (Imamura et al. 2002). A first antisense study of hepatic PLIN2 causes a decrease in hepatic steatosis, hypertriglyceridemia, and insulin resistance in obese mice without altering the level of expression of PLIN3 and PLIN4 (Imai et al. 2007). A second antisense study of PLIN2 shows the same type of result with a decrease in DAG and TAG in the liver as well as an improvement in insulin production (Varela et al. 2008). Finally, deletion of the PLIN2 exon 5 (Plin2 Δ 5) in mice causes resistance to obesity induced by a diet rich in fats indicating the role of PLIN2 in obesity and hepatic steatosis (Mcmanaman et al. 2013). The deletion of PLIN3 in mice induces cold tolerance (Lee et al. 2018), probably by regulating beige adipocyte formation and thermogenic activities. The deletion of PLIN4 leads to decreased expression of PLIN5 reducing lipid accumulation in the cardiac muscle (Chen et al. 2013). KO of PLIN6 is responsible for stopping the concentration of carotenoids in the droplets (Granneman et al. 2017).

Arthropoda

Research on LDs of *Arthropoda* was mainly carried out in the fruit fly *Drosophila melanogaster*. LD research began with two RNAi screens (Beller et al. 2008; Guo et al. 2008) showing that approximately 370 genes, or 1.5% of the expressed genome, were involved in LD physiology. A first proteomic study was conducted on the abdominal fat body, the fat tissue of the fly, which allowed the identification of 248 proteins (Beller et al. 2006). A second study was conducted on whole embryos (Cermelli et al. 2006). The proteomes of the LD of *D. melanogaster* have similarities with those of mammals. There are, for example, two members of the

Table 11.2 Analyses of LDs in representative prokaryotic and eukaryotic study models

Phylogeny	Proteobacteria	β -Proteobacteria	Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References
t2.1 [AU7]	Prokaryota		<i>Cupriavidus necator</i>	PhaC/PhaP/ PhaR/PhaZ	PHB	PHB granules	Bresan et al. (2016), Peoples et al. (1989), Schubert et al. (1988), Slater et al. (1988), Gemgross et al. (1993), Dennis et al. (1998), Tariq et al. (2015), Han et al. (2007)
t2.3		γ -Proteobacteria	<i>Marinobacter</i>		TAGs		Kalscheuer et al. (2007)
t2.4	Actinobacteria	Mycobacteriaceae	<i>Alcanivorax</i> <i>Mycobacterium tuberculosis</i> <i>Mycobacterium bovis</i> BCG <i>Mycobacterium avium</i>	PLIN2, PLIN3, ARL8B Tgs1, Tgs2, BCG1169c, BCG1489c, BCG1721	TAGs	Pathogenic bacteria	Menon et al. (2019), Kim et al. (2010), Caire-Brändli et al. (2014), Melo and Weller (2016), Luo et al. (2005), Low et al. (2010)
t2.7 [AU8]		Nocardiaceae	<i>Rhodococcus jostii</i> RHA1 <i>Rhodococcus opacus</i> PD630 <i>Rhodococcus ruber</i>	MLDS/Psp A LPD06283 Ribosomal protein L7	TAGs		Ding et al. (2012a, b) Kalscheuer et al. (2001), Chen et al. (2014) Kalscheuer et al. (2001)
t2.10 [AU9]							
t2.11							
t2.12	Chlamydiae		<i>Chlamydia trachomatis</i>	PLIN2, PLIN2, ACSL-3, ACSL-4, CGI-58	TAGs	Pathogenic bacteria	Saka et al. (2015)

Eukaryota	Unikonta	Opisthokonta	Metazoa	Cnidaria	<i>Euphyllia glabrescens</i>	SLDP	TAGs, sterol, WE, PE, PC, Lyso-PC	Analysis in multiple cell and tissue types	Peng et al. (2011)
				Chordata	<i>Cricetulus griseus</i>	PLINs, CGI-58, ATGL, HSL, CNX	TAGs/SE		Bartz et al. (2007), Bickel et al. (2009), Kimmel et al. (2010), Greenberg et al. (1991), Orlicky et al. (2008), Najt et al. (2014), Busell and Eliezer (2003), Hickenbottom et al. (2004), Kimmel and Szialryd (2016), Granneman et al. (2017), Mandard et al. (2004), Poulsen et al. (2012), Targett-Adams et al. (2005), Dalen et al. (2007), Edvardsson et al. (2006), Chawla et al. (2003), Schmuth et al. (2004), Tobin et al. (2006), Bindesbøll et al. (2013), Stenson et al. (2011), Langhi et al. (2014), Liu et al. (2004)

(continued)

t2.16 Table 11.2 (continued)

Phylogeny	Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References
12.17	<i>Mus musculus</i>				Wu et al. (2000), Brassamle et al. (2004), Cho et al. (2007), Kanshin et al. (2009), Blouin et al. (2010), Zhang et al. (2011), Ding et al. (2012a, b), Crunk et al. (2013), Goo et al. (2014), Yu et al. (2015), Yamaguchi et al. (2015), Wang et al. (2015), Kramer et al. (2018)
12.15	<i>Rattus norvegicus</i>				Turró et al. (2006), Larsson et al. (2012), Eichmann et al. (2015), Khor et al. (2014)
12.16	<i>Homo sapiens</i>				Fujimoto et al. (2004), Sato et al. (2006), Bouchoux et al. (2011), Moessinger et al. (2011), Beilstein et al. (2013), Dahlhoff et al.

t2.30 **Table 11.2** (continued)

t2.31	Phylogeny	Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References
t2.29	Amoebozoa	<i>Schizosaccharomyces pombe</i>		TAG/SE		Venkata and Shigeto (2012)
t2.30		<i>Rhodospiridium toruloides</i>				Zhu et al. (2015)
t2.31		<i>Dicystostellium discoideum</i>	plnA	TAGs/FFA		Du et al. (2013)
t2.32		<i>Trypanozoma cruzi</i>			LDs induction	D'Ávila et al. (2011)
t2.33	Excavata	<i>Leishmania amazonensis</i>				Lecoeur et al. (2013)
t2.34		<i>Leishmania major</i>				Rabhi et al. (2012, 2016)
t2.35	Viridiplantae	<i>Arabidopsis thaliana</i>	Seeds—SH oleosin, SL oleosin, U oleosin, pol-len—U oleosin, Caleosin, Stereoleosin	TAGs	OLDs (seeds, pollen, leaves)	Jolivet et al. (2004), Vernachova et al. (2011)
t2.36		<i>Brassica napus</i>				Kaavić et al. (2006), Jolivet et al. (2009)
t2.37		<i>Sesamum indicum</i>				Lin et al. (2005)
t2.38		<i>Jatropha curcas</i>				Popluechai et al. (2011), Liu et al. (2015)
t2.39		<i>Madia sativa</i>				Acevedo et al. (2012)
t2.40		<i>Camelina sativa</i>				Jolivet et al. (2013)
t2.41		<i>Gevuina avellana</i>				Acevedo et al. (2012)
t2.42		<i>Zea mays</i>				Tnami et al. (2011)

t2.43	<i>Arachis hypogea</i>					Jolivet et al. (2013)
t2.44	<i>Nicotiana tabacum</i>					Kretzschmar et al. (2018)
t2.45	<i>Brassica tapetum</i>	T-oleosin	TDGs		Tapetosome	Huang (2018)
t2.46	<i>Persea americana</i>	LDAP1, LDAP2, M-oleosin	TDGs		OLDs—mesocarp	Horn et al. (2013)
t2.47	<i>Allium cepa</i>	U-oleosin/caleosin	TDGs		OLDs—LDs cluster	Huang (2018)
t2.48	<i>Vanilla planifolia</i>	U-oleosin				
t2.49	<i>Aloe vera</i>	U-oleosin				
	<i>Hevea brasiliensis</i>	SRPP/REF	cis-1,4-polyisoprene		Rubber particles—NOLDs	Berthelot et al. (2014), Oh et al. (1999), Sando et al. (2009)
t2.50	<i>Capsicum annuum</i>	PAP/Fibrillin	Yes		Plastoglobules—NOLDs	Van Wijk and Kessler (2017)
t2.51	<i>Chlamydomonas reinhardtii</i>	MLDP	TDGs			Moellering and Benning (2010), Nguyen et al. (2011), James et al. (2011), Davidi et al. (2012)
t2.52	<i>Dunaliella bardawil</i>		TDGs			Javee et al. (2016)
t2.53	<i>Dunaliella salina</i>					
t2.55	<i>Dunaliella parva</i>					
t2.56	<i>Scenedesmus quadricauda</i>		TDGs			
t2.57	<i>Haematococcus pluvialis</i>	HOGP	TDGs/DCITS/PC/SQDG/DG DG		Pigments in LDs	Peled et al. (2011)
t2.58	<i>Chlorella sp.</i>	Caleosin	TDGs/DAG			Lin et al. (2012)
	<i>Lobosphaera incisa</i>		TDGs			

(continued)

t2.60 Table 11.2 (continued)

12.61	Phylogeny	Species	LD Proteome/ LD protein characterized	LD lipidome	Other information	References
12.59						Siegler et al. (2017), Bigogno et al. (2002)
12.60						Marlowe et al. (1984a), Marlowe et al. (1984b), Song et al. (2013), Shi et al. (2015), Shi (2019)
12.61						Vielser et al. (2012)
12.62						Nojima et al. (2013)
12.63						Yoneda et al. (2016), Lupette et al. (2019)
12.64						Watanabe et al. (2017)
12.65						Hu et al. (2017)
12.66						Pasaribu et al. (2014b), Jiang et al. (2014)
12.67						Bi et al. (2016)
12.68						LeKieffre et al. (2017), Le Cadre et al. (2006)
12.69						Le Cadre et al. (2006)

PLIN family (*DmPLIN1* and *DmPLIN2*) in the fat body abdominal, as well as the CGI-58 protein in the whole embryo proteome. As a result, *Drosophila* has been considered an interesting model for studying the role of LDs in the context of human pathologies. The conservation of LD-associated proteins is not complete, since PLIN3, PLIN4, and PLIN5 appear to be restricted to vertebrates. *DmPLIN1* is present only on the surface of LDs and is involved in promotion/prevention mechanisms for lipolysis (Bi et al. 2012). *DmPLIN1* contains four helices in the central region of the protein, capable of binding lipid compounds (Arrese et al. 2008; Lin et al. 2014). Mutant flies deficient of *DmPLIN1* have larger LDs. Single and giant LDs within the fat body of these mutants have also been found to confer an obesity phenotype (Beller et al. 2010). *DmPLIN2* is present in the cytoplasm and on the surface of LDs (Beller et al. 2010). *DmPLIN2* only plays a role in the prevention of lipolysis (Bi et al. 2012). *DmPLIN2* mutants have smaller LDs (Li et al. 2012). The double mutant fly *DmPLIN1/DmPLIN2* presents a marked reduction of LD size; however, LDs are still present in these mutants suggesting that there is an additional mechanism regulating lipid storage and lipolysis. *DmHSL* (Hormone-Sensitive-Lipase) is a lipase participating in lipolysis and interacting with *DmPLIN1* (Bi et al. 2012). A complementary analysis of the CG2254 protein identified in the proteome of LDs from *Drosophila* abdominal cells (Beller et al. 2006) showed that it was LD subset dehydrogenase 1 (*Lsdsh1*) (Thul et al. 2017).

Interestingly, the study of *Drosophila* LDs has highlighted an unsuspected role in the homeostasis of histones within the cell, a function that may be more frequent in eukaryotes than initially thought. Histones were first identified in the LD proteome of *Drosophila* embryos (Cermelli et al. 2006). These histones are not detected in the fat body abdominal proteome (Beller et al. 2006). These results were confirmed by a secondary study of the Jabba protein that co-immuno-precipitates with histones (Li et al. 2012). The presence of histones H2A, H2B, and H4 was also observed in the tobacco sphinx *Manduca sexta* (Soulages et al. 2012). Finally, it has recently been shown that histone H2Av was dynamically associated with *D. melanogaster* LD during cleavage and syncytial blastoderm stages (Johnson et al. 2018).

A recent study also investigated the protein ABHD4/ABHD5 (CGI-58) in *Drosophila* (Hehlert et al. 2019). The mutation of the *pummelig* (*puml*) gene encoding CGI-58 causes abnormal accumulation of TAG in mutant flies as well as a change in the FA profile of TAGs in Malpighian tubules (kidneys). In contrast to mammals, the *Drosophila puml* does not stimulate ATGL lipase activity (*brummer*) in vitro (Hehlert et al. 2019).

Proteomic studies of LDs have also been performed in other arthropod models such as the tobacco sphinx *Manduca sexta* (Soulages et al. 2012) or *Anopheles aquasalis* (Dias-Lopes et al. 2016), an important vector of *Plasmodium virax*, the main human malarial parasite in the Americas.

Nematoda

The nematode *Caenorhabditis elegans* is a worm constituting a popular study model for the study of apoptosis, embryonic development, and cellular aging. This

nematode has LDs measuring 1–1.5 μm in diameter in its intestine and in the hypodermis. Three proteomic studies of *C. elegans* LDs have been performed (Zhang et al. 2012; Na et al. 2015; Vrablik et al. 2015). The first shotgun proteomic analysis allowed the identification of 306 proteins of which 193 were known to be associated with mammalian LDs (Zhang et al. 2012). This first study identified the DHS-3 protein on the surface of the LD via a GFP fusion. A second proteome of the LD of *C. elegans* allowed the identification of 154 proteins of which 113 are common with the first proteome (Na et al. 2015). DHS-3 and MDT-28 are the two major proteins in *C. elegans* LD. The deletion of the *dhs-3* gene causes a decrease in the size of LDs as well as the amount of their TAG. The *mdt-28* mutant causes the formation of LD aggregations (Na et al. 2015). A third proteome compared the LD protein composition of a *C. elegans* wild type and high *daf-2* (e1370) fat mutant (Vrablik et al. 2015). Using a GFP construct, the ACS-4 protein, an acyl-CoA synthase, was localized at the surface of the *C. elegans* LD. It has long been thought that PLINs were lost in *C. elegans*. However, three isoforms of mammalian PLINs have been identified: PLIN-1a, PLIN-1b, and PLIN-1c. These isoforms have an N-terminal PAT domain, an amphiphilic region with imperfect helices, and four C-terminal helices (Chughtai et al. 2015). The *C. elegans* genome seems to also code for several sequences of the LD protein actors ABHD4 (*CeLid-1*) and ABHD5/*CGI-58* (*CeAbhd5.2*) (Lee et al. 2014; Xie and Roy 2015).

439 Fungi

Saccharomyces cerevisiae is a widely used model for studying lipid biology because the synthetic pathways in the ER are similar to those of plants and animals (Koch et al. 2014). LDs measure about 400 nm in this organism. The hydrophobic core of LDs of *S. cerevisiae* is composed of TAGs grouped in the center and surrounded by the steryl ester molecules (Leber et al. 1994; Czabany et al. 2008). There is also a minor proportion of squalene and sterols. The monolayer of phospholipids consists of PC, PI, PE, PA, and PS (Tauchi-Sato et al. 2002; Grillitsch et al. 2011). The proteome of the LD highlighted proteins that contribute to the synthesis of the hydrophobic core, such as sterol- $\Delta 24$ -methyltransferase, squalene epoxidase, and lanosterol synthetase (Leber et al. 1994; Athenstaedt et al. 1999) (Table 11.2). In *Pichia pastoris*, the polar lipid monolayer is mainly composed of PC and PE but there is also a lower proportion of PI, PS, PA, cardiolipin, lysophospholipids, and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) (Ivashov et al. 2013) (Table 11.2). Studies have also been conducted in other models such as *Yarrowia lipolytica* (Athenstaedt et al. 2006), *Mortierella alpina* (Yu et al. 2017), *Cryptococcus albidus* (Shi et al. 2013), *Trichosporon fermentans* (Shen et al. 2016), *Schizosaccharomyces pombe* (Noothalapati Venkata and Shigeto 2012), or *Rhodospiridium toruloides* (Zhu et al. 2015). Identified proteins comprise a majority of orthologues of *S. cerevisiae* and one can deduce from this analysis the enzymatic functions related to the synthesis of ergosterol, phospholipids, sphingolipids, but

also proteins involved in the metabolism of fatty acids and degradation of non-polar lipids (Table 11.2).

11.3.3.2 Amoebozoa

Only one study of LD was recorded for *Amoebozoa* in the model species *Dicystostelium discoideum*. *D. discoideum* is an amoeba living on dead leaves in forests, phagocytosing bacteria, or yeasts (Malchow et al. 1967). LDs of *D. discoideum* are composed of TAG, free fatty acid, and more than 10% of an unknown lipid (Table 11.2). Proteomic analysis of the LD of *D. discoideum* reveals 72 proteins including one perilipin (plnA) (Du et al. 2013). The expression of plnA in CHO cells allowed their localization to the surface of LDs (Miura et al. 2002). Fifteen lipid metabolism enzymes, 31 small GTPases belonging to the Rab family, eleven endoplasmic reticulum component proteins, and six cytoskeletal associated proteins were also identified.

In non-photosynthetic eukaryotes, LD studies support the general conservation of the architecture including some classes of perilipins, but with a striking diversification of proteins associated with the LDs likely associated with specialized functions. A role in histone homeostasis may be an important innovation in eukaryotes. The biogenesis process from the ER seems also conserved, with SEIPIN-associated machineries. Enzymes involved in the biosynthesis of TAGs, also lipases and proteins involved in lipolysis, such as CGI-58, seem to be the markers of LD evolution. However, the molecular function of CGI-58 seems to differ in the various clades of non-photosynthetic eukaryotes studied so far, a functional ‘flexibility’ which is also observed in photosynthetic eukaryotes (see below).

11.3.3.3 Photosynthetic Eukaryotes Originating from Primary Endosymbiosis

The acquisition of the primary chloroplast occurred when an unknown eukaryotic organism integrated a Gram-negative cyanobacterium (Petroutsos et al. 2014; Maréchal 2018). This event led to the emergence of a photosynthetic organelle with two membranes (inner and outer membranes of the envelope) called the plastid. Based on the machinery of photosynthetic pigments (Archibald and Keeling 2002; Petroutsos et al. 2014; Maréchal 2018), three lineages appeared. The green lineage of primary endosymbionts corresponds to *Viridiplantae*. This lineage includes *Chlorophyta* (“green algae”) and *Streptophyta* (commonly called “plants”). The photosynthetic machinery is composed of chlorophyll a and b. The red lineage of primary endosymbionts consists of *Rhodophyta* or “red algae”. These organisms have chlorophylls a and c associated with phycobilin. The ‘blue’ lineage of primary endosymbionts corresponds to Glaucocystophytes (*Cyanophora paradoxa*), having a chloroplast with a residual cell wall rich in peptidoglycans. Chlorophyll a is associated with phycocyanin and allophycocyanin. Primary endosymbiosis has

long been considered a unique event during evolution. The study of *Paulinella chromatophora* (a photosynthetic amoeba) has shown that a second, more recent, primary endosymbiosis event (60–100 million years ago) occurred between cyanobacteria and an amoeba (Maréchal 2018). This endosymbiosis led to the formation of an organelle also limited by two membranes, called the chromatophore. In contrast to non-photosynthetic eukaryotes, in which FA biosynthesis occurs in the cytosol, FAs are synthesized in the stroma of the chloroplast and then exported to the cytosol. On the one hand, chloroplasts contain LD, called plastoglobules. On the other hand, the plastid appears to play a role in the production of TAG and biogenesis of cytosolic LDs in some of the lineages of photosynthetic eukaryotes. Stronger cooperation of the ER and plastid in LD formation may therefore be an important innovation in these primary endosymbionts. This may also be related to the loss of perilipins and the emergence of specific LD-associated proteins. Our understanding of LD evolution in primary endosymbionts is mainly based on analyses performed in Chlorophyta and land plants (Embryophyta, mostly in Angiosperms).

Chlorophyta

Chlamydomonas reinhardtii is a green alga that accumulates oils in the form of LDs following environmental stresses such as a nitrogen deficiency or an increase in salinity. A proteomic study of LD performed in *C. reinhardtii* highlighted a Major Lipid Droplet Protein (MLDP) of 27 kDa (Moellering and Benning 2010; James et al. 2011; Nguyen et al. 2011). The phenotype of a *mldp* mutant suggests that MLDP is involved in the regulation of LD size (Moellering and Benning 2010). A 33 kDa homolog of MLDP was also found in *Haematococcus pluvialis* (Peled et al. 2011) as well as in three *Dunaliella* species (Davidi et al. 2012). MLDP was also detected in *Scenedesmus quadricauda* during salt stress or nitrogen deficiency (Javee et al. 2016). MLDP orthologues are also present in several species of Chlorophyta: *Volyox carteri*, *Haematococcus pluvialis*, *Dunaliella salina*, *Coccomyxa* sp., *Chlorella variabilis*, *Polytomella parva*, *Prototheca wickerhamii*, and *Micromonas pusilla* CCMP1545 (Goold et al. 2015). A 28 kDa caleosin protein was shown to be the major protein in the LD of *Chlorella* sp. (Lin et al. 2012). The size of LDs of *Chlorella* can reach 3 µm (Lin et al. 2012). The caleosin localization was specifically determined on the surface of the LD by immunostaining with gold beads (Pasaribu et al. 2014a). In these studies, caleosins first characterized in plants (see below) seem to be conserved LD-associated proteins.

Plantae

Plants accumulate LDs in both vegetative and reproductive tissues (Chapman et al. 2012). The involvement of LDs in the physiology and development of plants are currently little known. The LD proteins of plants are divided into three functional

groups: (1) oleosins; (2) caleosins, steroleosins, and dioxygenases; and (3) the proteins associated with the LD.

Oleosins were the first proteins characterized on the surface of LDs of *Zea mays* seeds (Qu et al. 1986; Vance and Huang 1987). By their small molecular weight (15–26 kDa), oleosins are very abundant proteins on the surface of LDs of plants. Structurally, the oleosins are divided into three portions: (1) a short and amphiphilic N-terminal peptide, (2) a C-terminal amphiphilic peptide of varying length, and (3) a hydrophobic pin of nonpolar amino acids penetrating the monolayer of phospholipids on the surface of the LD (Huang 2018). The N- and C-terminal peptides form receptor binding lipases and other proteins involved in TAG degradation (Huang and Huang 2015). The 72 amino acid pin is a specificity of oleosins (Kory et al. 2016), thus differing from major LD proteins of mammals (PLINs 1-6) or bacteria (Phasin). The pin is also divided into three portions, consisting of two 30-amino acid arms connecting a loop consisting of three prolines (P) and one serine (S) forming a structure called the “Proline Knot” inserted into the hydrophobic core [19, 229]. The secondary structure of the loop has not been defined yet. Seventeen genes code for the oleosins in *Arabidopsis*: five in the seed, three jointly in the seeds and pollen grains, and nine in the floral cells of the tapetum [230].

In a bioinformatic study, oleosins could be classified into six major lineages (Huang and Huang 2015): the primitive lineage evolving from green algae to Filicophyta (ferns), the universal lineage (U oleosin) for which genes are present, and Bryophyta (mosses) to higher plants. The universal U line then evolved to specialize in particular structures such as seed-specific oleosins with the Low and High Molecular Weight Seed Oleosin (SH) lines in Angiosperms. Oleosins also specialized in Brassicaceae with the tapetum T line and the M line for oleosins in the Lauraceae mesocarp (avocado) (Kilaru et al. 2015).

Caleosins, stereoleosins, and dioxygenases are grouped into a single cluster because they have a common enzymatic function in the stress response (Huang 2018). Caleosins are enzymes that have been found in microsomes (Frandsen et al. 1996). Caleosins, like oleosins, have a hydrophobic as well as a “proline knot” motif (Huang 2018). A recent study showed that two of the hairpin prolines (P116 and P125) were not essential for LD binding (Müller et al. 2016). Caleosins have an N-terminal EF hand-type calcium binding motif (Chen et al. 1999), a peroxygenase activity (Hanano et al. 2006), and several phosphorylation sites. The genome of *Arabidopsis thaliana* codes for eight caleosins expressed in different structures (Shimada and Hara-Nishimura 2015).

Steroleosins (sterol dehydrogenases) have only two structural domains: a hydrophobic N-terminal region and a C-terminal region having a sequence close to the mammalian hydroxysteroid dehydrogenase (HSD) domain. Steroleosins also have a semi-conserved hydrophobic pin similar to that of oleosin, but of a size similar to that of caleosin (Huang 2018). They are also class I proteins such as oleosins and caleosins (Kory et al. 2016). In contrast to oleosins and caleosins, steroleosins do not have a “proline knot” motif but a “proline knob” (Chapman et al. 2012). Steroleosins are particularly studied because they are capable of converting sterols into brassinosteroids (Baud et al. 2009), a class of phytohormones.

Dioxygenases (α -DOX) have also recently been found to be associated with LDs in leaves of senescent *Arabidopsis thaliana* cells (Shimada et al. 2014). *A. thaliana* has two homologs of these dioxygenases (At α -DOX1 and At α -DOX2). At α -DOX1 is localized on LDs of leaves and At α -DOX2 is located in the ER (Shimada et al. 2014). These enzymes produce an oxylipin (2-HOT) from α -linolenic acid (18:3). These molecules participate in defense mechanisms in response to biotic and abiotic stresses (Shimada and Hara-Nishimura 2015).

Not all LD proteins listed above belong to the same LDs. Indeed, in plants, cytosolic LDs are divided into two groups: oleosin-based lipid droplets (OLDs) and non-oleosin-based lipid droplets (NOLDs) (Laibach et al. 2015).

Seeds are the most studied structure for the understanding of OLDs because they are able to accumulate TAGs in the form of LDs reserve to support germination after the end of the dormancy phase (Huang 1996). In special cases such as jojoba seed (*Simmondsia chinensis*), LDs can contain cerides (Yermanos 1975). These LDs are small (between 0.5 and 1.5 μ m) conferring a large surface area per unit of TAGs, facilitating the binding of lipases during germination (Huang and Huang 2015). Numerous proteomic studies have been performed in plant models: *Brassica napus* (Katavic et al. 2006; Jolivet et al. 2009), *Arabidopsis thaliana* (Jolivet et al. 2004; Vermachova et al. 2011), *Sesamum indicum* (Lin et al. 2005), *Jatropha curcas* (Popluechai et al. 2011; Liu et al. 2015), *Madia sativa* (Acevedo et al. 2012), *Gevuina avellana* (Acevedo et al. 2012), *Zea mays* (Tnani et al. 2011), *Camelina sativa* (Jolivet et al. 2013), or *Arachis hypogaea* (Jolivet et al. 2013). These proteomic analyses show that LDs are covered with oleosins with a minor presence (less than 5%) of caleosin and steroleosin (Chapman et al. 2012; Murphy 2012). Oleosins are involved in regulating the size and stability of LDs of seeds (Chapman et al. 2012). LDs have also been characterized in the tapetum cells of the anther (Hsieh and Huang 2004) and in pollen grains and pollen tubes (Kretzschmar et al. 2018). A focused study on PUX10 (Plant UBX Domain-containing Protein 10) whose localization was confirmed by fusion with enhanced GFP (eGFP) on the surface of LDs during embryonic development, seed germination, and pollen tubes, showed that PUX10 recruits by its UBX domain an AAA-type ATPase Cell Cycle 48 (CDC48) that facilitates the transfer of polyubiquitinated protein to the 26S proteasome (Kretzschmar et al. 2018).

LDs are also present in *Arabidopsis thaliana* leaves. The number of LDs is very low in healthy leaves. LDs accumulate more in the leaves in the senescence phase (Shimada et al. 2015) with a variable size of 1–18 μ m (Lersten et al. 2006). In particular, the expression of *A. thaliana* caleosin-3 as well as At α -DOX1 increases during senescence (Shimada et al. 2014). Proteomic analysis of the LD of aging leaves of *A. thaliana* was performed: 28 proteins including 9 enzymes involved in the secondary defense metabolism of the plant were identified (Brocard et al. 2017). The analysis also revealed the presence of the Small Rubber Particle 1 (AtSRP1) protein. Functional analysis of AtSRP1 reveals that this protein modulates the expression of caleosin-3 in aging leaves. In addition, overexpression of AtSRP1 induces an increase in 18:3 enriched TAG accumulations from galactolipid recycling of thylakoids (Brocard et al. 2017).

LDs of the fruit mesocarp can reach sizes of 10–20 μm , placing them at the top of the ranking of the largest observable LDs in eukaryotic cells (Horn et al. 2013). Proteomic analysis of the mesocarp of the avocado (*Persea americana*) allowed the identification of two LDAP1 and LDAP2 associated proteins (Lipid Droplet-Associated Proteins 1 and 2), which also showed homologies of sequences with Small Rubber Particle Proteins (SRPP) (Horn et al. 2013). Type M oleosins specific to the *Lauraceae* family have been described in avocado (Huang 2018).

NOLDs include two special cases: rubber particles and plastoglobules. More than 20,000 species of higher plants can accumulate rubber particles within their vegetative organs (Hagel et al. 2008). *Hevea brasiliensis* is the main source of latex used by humans. Latex is a colloidal white suspension composed of rubbery and non-rubbery particles, organelles, proteins, lipids, carbohydrates, and minerals. These particles have a hydrophobic core consisting of *cis*-1,4-polyisopropene surrounded by a monolayer of phospholipids in which proteins are bound (Berthelot et al. 2014). The proteome of the rubber particles revealed two major proteins: the SRPP and the Rubber Elongation Factor (Sando et al. 2009).

Finally, plastoglobules are special LDs synthesized inside the chloroplast (Br  h  lin et al. 2007). Plastoglobules are continuous with the outer monolayer of thylakoids in higher plants, which is supposed to facilitate the exchange of metabolites (Van Wijk and Kessler 2017). Plastoglobules have been less studied than cytosolic LDs. Analyses support that they have a hydrophobic core containing three classes of molecules: (1) neutral lipids (TAGs, phytol esters, and free fatty acids), (2) tocopherols and quinones (α -tocopherol, plastoquinol-9, plastochromanol-8, and Vitamin K₁), and (3) linear carotenoids (lycopene), cyclic carotenoids (lutein and xanthophylls), and carotenoid esters (Van Wijk and Kessler 2017). These molecules are surrounded by a monolayer of amphiphilic lipids (monogalactosyldiacylglycerol, MGDG; digalactosyldiacylglycerol, DGDG; sulfoquinovosyldiacylglycerol, SQDG) in which proteins are embedded. Proteomic analyses of several study models (the chromoplast of red pepper *Capsicum annuum* and the green microalgae *Dunaliella bardawil* and *Chlamydomonas reinhardtii*) have allowed the identification of about 30 proteins (Kreimer 2009; Davidi et al. 2015). The protein mainly represented on the surface of plastoglobules is Plastid-lipid Associated Protein, Fibrillin (PAP/Fibrillin) (Youssef et al. 2010). This 30-kDa protein does not have transmembrane segments.

11.3.3.4 Photosynthetic Eukaryotes and Non-photosynthetic Relatives Originating from a Secondary Endosymbiosis

Secondary endosymbioses are events that have occurred several times during the evolution of eukaryotes (Petroutsos et al. 2014). Two main types of lineages have emerged as a result of these evolutionary events: (1) green lines resulting from the integration of a green alga within an unknown heterotrophic eukaryotic organism, leading to the appearance of *Euglenozoa* and Chlorarachniophytes and (2) the red

lines resulting from the integration of a red alga inside a eukaryotic organism forming the polyphyletic group of *Chromalveolata*.

Organisms from the Green Lineage: *Euglenozoa*

At least two independent events of secondary endosymbiosis between a green alga and an unknown heterotrophic eukaryotic organism led to the appearance of *Euglenozoa* and Chlororarachniophytes (Petroutsos et al. 2014; Füssy and Oborník 2018). Chlororarachniophytes (*Bigelowiella natans*) contain a four-membrane chloroplast and a residual nucleus, called the nucleomorph, located between the two most internal and external membranes of the chloroplast (Petroutsos et al. 2014). To our knowledge, no data are available on LD formation in Chlororarachniophytes. *Euglenozoa* comprises photosynthetic species with a chloroplast bounded by three membranes but also parasitic species devoid of any chloroplast (Petroutsos et al. 2014). Several studies report that parasitic organisms such as *Trypanosoma cruzi*, the agent of Chagas disease (D'ávila et al. 2011), are capable of inducing the formation of large LDs in macrophages. In photosynthetic organisms, *Euglena gracilis* is a microalga living in freshwater, interesting for the research of alternatives to petroleum resources because it accumulates wax esters inside LDs in nitrogen starvation. These wax esters come from the conversion of a crystalline β -1,3-glucan, paramylon. Wax esters can be used as fuel for aviation but also as biofuel after refining (Guo et al. 2017). To our knowledge, there is no detailed study of the structure of the LD of *E. gracilis*.

Organisms from the Red Lineage: *Chromista/Chromalveolates*

Apicomplexa (Containing a Non-photosynthetic Plastid)

Apicomplexa is a phylum grouping unicellular parasitic organisms responsible for many diseases in metazoans such as malaria or toxoplasmosis. As noted previously with pathogenic bacteria, parasitic *Euglenozoa* or HCV parasites, these organisms are also able to divert the lipid metabolism of the host by inducing the formation of LDs or by modifying their architecture. One of the most commonly studied *Apicomplexa* models is *Toxoplasma gondii*, the toxoplasmosis agent. *T. gondii* replicates in mammalian cells in a parasitophorous vacuole. *Toxoplasma* induces an increase in the catalytic activity of the host DGAT, which leads to diversion of lipid metabolism toward TAGs and guarantees the import of FAs (Hu et al. 2017).

Dinophyta

Dinophyta or Dinoflagellates are photosynthetic protists, but also mixotrophic and heterotrophic, with two flagella allowing them to move (Sardet 2013). There are symbiotic forms of Dinoflagellates, especially with corals. LDs are present in hosts

and symbionts of coral-dinoflagellate endosymbiosis (Kellogg and Patton 1983). The first proteomic analysis of the gastrodermale LDs of *Euphyllia glabrescens* (*Cnidaria*) revealed the presence of 42 proteins involved in the metabolism of lipids and proteins as well as in the response to some stresses (Peng et al. 2011). A second proteomic analysis of LDs of *Symbiodinium* sp. associated with coral tentacles of *Euphyllia glabrescens*, highlighted a 20 kDa Symbiodinium Lipid Droplet Protein (SLDP) that plays a role in the structural and functional stability of the LD (Pasaribu et al. 2014b). Proteomic analysis of LDs of isolated *Symbiodinium* spp. has also been performed (Jiang et al. 2014). This study did not reveal the presence of SLDP but of several proteins involved in lipid metabolism (Sterol transfer family protein), cell signaling (14-3-3 protein, ADP ribolysis factor), stress response (HSP90), and energy metabolism (ATP synthase F1 subunit α , GTP binding protein).

Haptophyta

Haptophyta include photosynthetic species forming extracellular shells of calcium carbonate (coccolithophores' coccospheres) and non-calcified cell walls (Cavalier-Smith 1986). A proteomic study was carried out in the non-calcareous haptophyta *Tisochrysis lutea*, formerly *Isochrysis* aff. *galbana* (Bendif et al. 2013). This microalga is interesting for the aquaculture industry because it is enriched in DHA (Hubert et al. 2017). It is also known to accumulate a particular neutral lipid in place of TAGs: an alkenone, a very long chain ketone (C_{37} to C_{40}), within an alkenone body (AB) (Marlowe et al. 1984a, b). The purification of LDs highlighted the presence of 74.2% of alkenone (C_{37} and C_{38}), 24.6% of other lipids, and 1.2% of alkene. Proteomic analysis revealed the presence of 514 proteins on the surface of *T. lutea* AB, of which three are predominant: a V-ATPase identified previously in a proteomic analysis in nitrogen deficiency (Song et al. 2013), an SPFH (Stomatin/Prohibitin/Flotillin/HflK) domain-containing protein, localized in the endoplasmic reticulum, associated with a lipid raft and a hypothetical protein (Shi et al. 2015). With the exception of SPFH, the transition between a rich medium and a low nitrogen medium causes an increase in the expression of the target genes encoding *T. lutea* AB proteins (Shi 2019).

Heterokonta

Heterokonta (or Stramenopiles) constitute a superphylum in the *Chromista* kingdom (Cavalier-Smith 2018). Five proteomic studies were performed in four model species: *Nannochloropsis oceanica* (Vieler et al. 2012), *Phaeodactylum tricornutum* (Yoneda et al. 2016; Lupette et al. 2019), *Fistulifera solaris* (Nojima et al. 2013), and *Aurantiochytrium limacinum* (Watanabe et al. 2017). The first study conducted with *N. oceanica* (Vieler et al. 2012) revealed a 16.8 kDa Lipid Droplet Surface Protein (LDSP) by proteomic analysis. This protein is present in the six species of *Nannochloropsis* with functional features close to plant oleosins as well as MLDP of *Chlamydomonas reinhardtii*. The second proteomic study in diatoms concerns the pennate diatom *Fistulifera solaris* (Nojima et al. 2013). The authors did not detect

any major band and the purification seems to involve protein contaminations. By subtracting the protein purification proteins from the soluble fraction, the authors identified fourteen candidate proteins. By searching for conserved domains, one of the identified proteins has a quinoprotein-alcohol dehydrogenase-like domain detected in the proteome of LDs of *Camelina sativa* seed (Jolivet et al. 2013) but has no hydrophobic domain. This protein of 506 amino acids was named DOAP1 (Diatom-Oleosome-Associated-Protein 1). Based on a fusion between GFP and the N-terminal signal sequence of DOAP1 (Maeda et al. 2014), this protein was initially present in the ER. The third model characterized is the pennate diatom *Phaeodactylum tricornutum*. A first proteomic study allowed the identification of five major proteins on the surface of the LD of *P. tricornutum*, including the Stramenopile LD Protein (StLDP) (Yoneda et al. 2016). This class I protein was localized to the surface of the LD by fusion with a GFP protein (Yoneda et al. 2018). By optimization of the *P. tricornutum* LD purification protocol, a second study was carried out in this organism (Lupette et al. 2019). The proteome of the LD is composed of 86 proteins including most notably the LD-protein StLDP, metabolic actors, organelle membrane-associated proteins, proteins implicated in the treatment of genetic information, or chaperones involved in protein quality control. The hydrophobic core is only made of TAG surrounded by a monolayer of polar lipids consisting of PC, SQDG, and two molecular species (20:5–16:1 and 20:5–16:2) of diacylglycerylhydroxymethyltrimethyl- β -alanine (DGTA), a betaine lipid. A sterol probably located in the polar lipid monolayer, brassicasterol, was also detected and specific enrichment of β -carotene has been observed (Lupette et al. 2019). A last proteomic study was recently conducted on the model *Aurantiochytrium limacinum* F26-b, a non-photosynthetic unicellular microalga classified as *Heterokonta*. This microalga is particularly studied for its biotechnological potential because it is a species rich in DHA (22:6) (Dellero et al. 2018a, b; Morabito et al. 2019). A proteomic study of the LD fraction of *A. limacinum* F26-b identified a Thraustochytrid-specific Lipid Droplet Protein 1 (TLDP1) (Watanabe et al. 2017). A mutant of *tlp1* shows a decrease in the amount of TAG and the number of lipid droplets per cell. However, larger and irregular LDs are observed in this mutant. TLDP1 may regulate the accumulation of TAGs as well as the size and number of LDs in *A. limacinum* F26-b (Watanabe et al. 2017).

Based on all these analyses of secondary endosymbionts, it appeared that major LD proteins are specific to distinct clades and that their origin needs to be traced in evolution. Lipids associated with the few LDs analyzed to date sometimes include plastid lipids together with ER lipids, suggesting cooperation of these two organelles in LD biogenesis. Detection of lipases, often associated with mitochondrial and/or lysosomal processes, further suggests a conserved role of mitochondria and lysosomes in the mobilization of FAs deriving from TAG stored in cytosolic LDs.

11.4 Lipid Droplets Are Dynamic Structures

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LDs are dynamic subcellular structures, most often transient, rarely stable. Their development includes an intense phase of biosynthesis of the hydrophobic molecules that constitute its core (anabolism) and the biosynthesis and the arrangement of the barrier located at the periphery (a monolayer of polar lipids, most often phosphoglycerolipids, and proteins, either embedded in this monolayer or associated more or less transiently).

11.4.1 Anabolism of PHA and TAG

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11.4.1.1 PHA Biosynthesis

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PHA biosynthesis routes have been recently reviewed by Możejko-Ciesielska and Kiewisz (2016). Depending on the length of the PHA chain, several pathways exist. For short-chain PHA, a direct pathway involves three enzymes: a β -ketothiolase (PhaA), an acetoacetyl-CoA reductase (PhaB), and a PHA synthase (PhaC). For medium chain PHA, several pathways are possible: (1) a synthetic pathway involving β -oxidation resulting in the formation of R-3-hydroxyacyl-CoA that is converted by PhaC to PHA; (2) a synthetic route involving elongation of acetyl-CoA, via de novo synthesis of fatty acids; and (3) a synthetic route generating precursors of 3-hydroxyacyl-CoA.

11.4.1.2 TAG Biosynthesis

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The hydrophobic core of LDs described in eukaryotes contains predominantly TAGs. The synthesis of TAGs can be carried out by two main pathways at the level of the endoplasmic reticulum (the so-called “eukaryotic” pathway): (1) an acyl-CoA dependent pathway commonly referred to as the Kennedy pathway (Fig. 11.2), (2) an acyl-CoA independent pathway involving phosphatidylcholine (PC) as an acyl donor, and (3) a specific route of synthesis of TAGs in the chloroplast.

11.4.2 Biogenesis of the Lipid Droplet

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11.4.2.1 Biogenesis of PHA Granules in Prokaryotes

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There are currently two models for the *in vivo* formation of PHA granules in prokaryotes: (1) a model based on the formation of micelles of PHA synthases (PhaC) and (2) a model based on the budding of PHA granules from the cytoplasmic membrane (Pötter and Steinbüchel 2006). The model based on the formation of

micelles of PHB synthases is currently the most accepted (Haywood et al. 1989). We do not know if any of these processes has been transferred to a eukaryotic system.

11.4.2.2 Biogenesis of Cytosolic TAG Droplets in Eukaryotes

The general principles of the biogenesis mechanism seem to be similar in non-photosynthetic and photosynthetic organisms (Fig. 11.2—TAG synthesis). The first step consists of the synthesis of TAG at the level of the ER, through the Kennedy pathway (see Sect. 11.3.1.2). Following the synthesis of TAGs, a “lens” of neutral lipids (TAGs and sterol esters) develops within the bilayer of the ER at particular nucleation sites (Fig. 11.2—Nucleation) (Pol et al. 2014; Wilfling et al. 2014). A number of pre-droplets are formed in the ER from freely diffusing TAG molecules, but only a small proportion of them will ultimately form a lipid droplet. The lifespan of these lipid pre-droplets is estimated at a few milliseconds (Khandelia et al. 2010). This phenomenon leads to an energy optimum called a nucleation barrier (Thiam and Forêt 2016). The homogeneity of the phospholipid bilayer of the reticulum is also an important parameter to take into account the success of nucleation. Indeed, on a uniform bilayer, the nucleation of LDs could occur randomly. However, the phospholipid bilayer of the ER is heterogeneous in its biochemical composition. There are therefore specific sites where the nucleation energy is lower. Nucleation can be favored by four different phenomena: (1) the curvature of the membrane developing hydrophobic defects favorable to the accumulation of TAG, (2) the synthesis sites of TAGs defined by the presence of DGATs, or other synthetic enzymes such as PDATs, (3) the presence of proteins (SEIPIN, FIT, PLINs, ACSL) inducing curvature in decreasing the bending energy of the membrane, and (4) the presence of proteins and/or lipids interacting with TAGs (Thiam and Forêt 2016).

Following the formation of this lens, budding (Fig. 11.2—Budding) of the LD occurs on the cytoplasmic side of the ER (Walther et al. 2017). This polarized budding of LDs follows an unknown mechanism. In humans, it has been proposed that an ER protein, FIT2 (Fat-inducing transcript 2), was involved in the polarization of budding (Choudhary et al. 2016). There are currently several models concerning the growth of the LD, including the coalescence and Ostwald ripening (Thiam et al. 2013b). Coalescence is a physical mechanism corresponding to the fusion of two identical substances (in our case, the fusion of two LDs). When an emulsion of oil and water is vigorously mixed, the fusion of LDs with each other is observed to form a giant lipid droplet reflecting the separation between the oil and the water. Ostwald ripening is a destabilization mechanism corresponding to the gradual disappearance of small LDs from an emulsion to give way to larger LDs (Thiam et al. 2013b). This mechanism begins with the transfer of TAG molecules from small LDs to larger ones. The direction of the transfer is dictated by the pressure difference of Laplace (Thiam et al. 2013b; Thiam and Forêt 2016).

An increase in the contact angle between the LD and the ER bilayer causes fission of the LD (Fig. 11.2—Fission). This mechanism also involves proteins on the surface of the reticulum. An interesting candidate is the SEIPIN protein located at

the level of the ER with two transmembrane domains and a luminal loop (Lundin et al. 2006). SEIPIN is involved functionally in the initial stages of LD formation into generating nascent structures or participating in their maturation in mature LD (Wang et al. 2016). A morphological screen of LDs of *Saccharomyces cerevisiae* shows that the homolog Fld1 of SEIPIN is necessary for the formation of “normal” droplets (Fei et al. 2008). This protein also participates in the stabilization of the contact sites between the ER and the LD (Salo et al. 2016). A recent study also proposes that the SEIPIN protein and the LDAF1 (Lipid Droplet Assembly Factor 1) protein form an oligomeric complex of approximately 600 kDa in the ER bilayer determining the LD formation sites (Chung et al. 2019). SEIPIN is also involved in the regulation of TAG synthesis: it interacts notably with GPAT, AGAT2, and lipins (phosphatidate phosphatases recruited for the synthesis of DAG, serving as the substrate to the production of TAG). A single Seipin gene is detected in animals and fungi studied so far, whereas three homologs (SEIPIN1, 2, and 3) were reported in higher plant models (Taurino et al. 2017). SEIPINS are divided into two monophyletic groups in plants according to their degree of functional specialization (Cai et al. 2015). A functional genetic study shows that double and triple mutations cause the accumulation of larger LDs in *Arabidopsis* (Taurino et al. 2017). An ortholog of SEIPIN was also detected in the diatom *Phaeodactylum tricornutum* (Lu et al. 2017). The overexpression of SEIPIN in *P. tricornutum* causes an increase in the size of LDs, the amount of neutral lipids (57% increase), and the proportion of saturated fatty acids (16:0) (Lu et al. 2017).

LDs can detach from the ER, forming a population of initial LDs (iLD) measuring 400–800 nm in diameter (Walther et al. 2017). These LDs can be subsequently converted, according to an unknown mechanism, into a new population of LDs called expanding lipid droplets (eLDs) (Walther et al. 2017). This conversion requires the acquisition of an independent TAG synthesis machinery carried out by GPAT4 and DGAT2 (Kuerschner et al. 2007; Stone et al. 2009; Wilfling et al. 2013). These two proteins initially present in the ER are relocated to the LD (Wilfling et al. 2013).

Although the conversion mechanism is unknown, there is still evidence that the vesicular machinery ARF1/COP1 (ADP Ribosylation Factor 1/Coat Proteins type I) is involved in this process. In particular, it has been shown in an in vitro system that the ARF1/COP1 machinery allows the synthesis of nano LDs of 60–80 nm (Thiam et al. 2013a). COP1 is a complex composed of seven subunits (α , β , β' , γ , ϵ , δ , ζ) responsible for the retrograde vesicular transport of the Golgi apparatus to the ER as well as intra-Golgi transport (Beck et al. 2009; Jackson 2014). ARF1 is a member of the family of GTP-binding proteins of the Ras superfamily (small GTPases). ARF1 is localized in the Golgi apparatus and was initially shown to act in intra-Golgi transport. The role of the ARF1/COP1 system in LD dynamics needs therefore to be clarified further compared to that in Golgi dynamics.

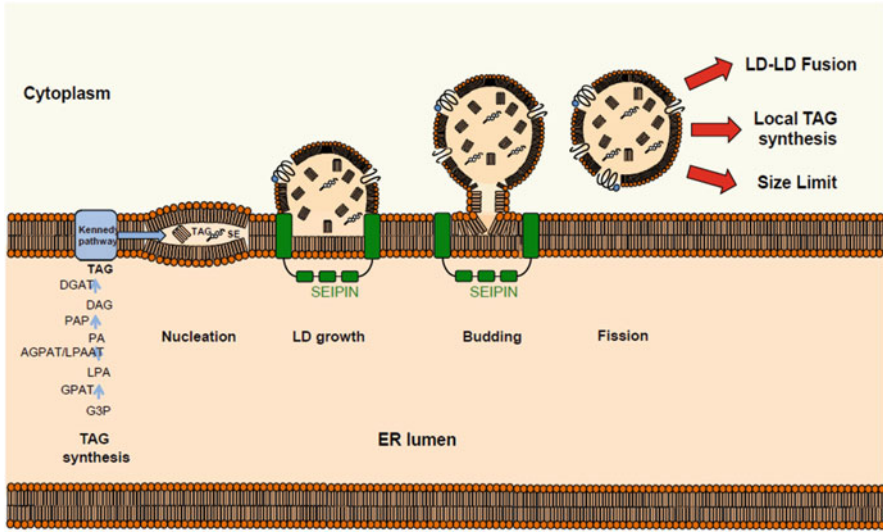


Fig. 11.2 Principles of lipid droplet biogenesis in eukaryotes

11.4.3 Catabolism of Lipid Droplets

The catabolism of LDs corresponds to all the degradation reactions of the LD architecture components, e.g. the lipids and hydrophobic components in the core of the LDs and the proteins especially *via* the ERAD pathway and the 26S proteasome.

11.4.3.1 Degradation of PHA in Prokaryotes

The degradation of PHA granules is carried out by a PHA depolymerase (PhaZ) (Uchino et al. 2008). PhaZ plays a role similar to acyl lipases in eukaryotes (see below). At least two degradation pathways of PHA granules exist: (1) an extracellular pathway in bacteria capable of secreting depolymerases in the environment and (2) an intracellular route, less well known to date. Seven intracellular PHB depolymerases (PhaZ1 to PhaZ7) as well as two PHB hydrolases (PhaY1, PhaY2) have been described in the *R. eutropha* H16 model (Abe et al. 2005; Uchino et al. 2008; Sznajder and Jendrossek 2014). However, PhaZ1 appears to be the only active PHB depolymerase in *R. eutropha* H16 (Sznajder and Jendrossek 2014). PhaZ1 presents a cysteine in the catalytic site of the “box” lipase. The thiolysis of PHB granules into (R)-3-hydroxybutyryl-CoA by PhaZ1 has been demonstrated (Eggers and Steinbüchel 2013). Localization of PhaZ1 has been confirmed on the surface of PHB granules by fusion with eYFP (Uchino et al. 2008). A mechanistic model of PHB granule degradation was proposed by Eggers and Steinbüchel (2013).

11.4.3.2 Degradation of TAG in Eukaryotes

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Fatty Acid Mobilization

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Multiple processes of TAG hydrolysis have been described in different eukaryotic clades. 922 923

In mammals, lipolysis involves different protein actors. The CGI-58 protein interacts with the terminal end of PLIN1a (PLIN1 splice variant) on the surface of the LD in fat cells (Subramanian et al. 2004). The adipose triacylglycerol lipase (ATGL) is also localized at the level of the LD. Before the lipolysis process takes place, the enzymatic activity of ATGL is very low. The majority of ATGL proteins are located in the cytosol before lipolysis occurs. The ATGL activity is inhibited by the G0/G1 switch protein 2 protein (G0S2), attenuating lipolysis in a dose-dependent manner (Schweiger et al. 2012). Lipolysis can be initiated by β -adrenergic stimulation (physical exercise, nutritional deficiency). Adenylate cyclase is activated by a heterotrimeric G protein resulting in the accumulation of cAMP, subsequently activating protein kinase A (PKA) (Viswanadha and Londo 2008). PLIN1 is then phosphorylated by PKA (Granneman et al. 2009). The association of ATGL with LDs is considered to depend on the ARF1/COP1 vesicular machinery (Soni et al. 2009). PKA also phosphorylates CGI-58 thus promoting the binding of CGI-58 to ATGL (Sahu-Osen et al. 2015). This process activates ATGL leading to the hydrolysis of TAGs into DAG and FAs. A Hormone-Sensitive Lipase (HSL) can be activated by phosphorylation: the HSL then binds to the LD, at the level of phosphorylated PLIN1, allowing the conversion of DAG into monoacylglycerol (MAG) and FAs (D'andrea 2016). The HSL/PLIN1 interaction is enhanced by phosphorylation by PKA on several residues of PLIN1: Ser81, Ser222, and Ser276. A monoglyceride lipase (MGL) then completes the release of the last FA and the glycerol backbone. The three released FAs are then transferred to the mitochondria (D'andrea 2016). 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946

LDs are strongly reorganized during lipolysis following a process that reduces their size and increases the surface/volume ratio and accessibility to lipases. Two models exist for the formation of these lipid microdroplets: (1) fragmentation of a giant LD (Moore et al. 2005) and (2) capture of released FAs, which are potentially toxic following lipolysis (Paar et al. 2012). A fat-specific-protein of 27 kDa (FSP27 also known as CIDEC for cell death-inducing DNA fragmentation factor alpha-like effector c) binds to ATGL at the surface of LDs inhibiting the lipolysis mechanism by blocking access of CGI-58 to ATGL. A reduction in FSP27 expression by siRNAs in a 3T3-L1 mouse line causes the formation of multiple small LDs as well as an increase in lipolytic activity (Nishino et al. 2008). FSP27 proteins are involved in lipid transfer mechanisms between LDs favoring mechanisms of fusion, growth, and enlargement (Gong et al. 2011; Jambunathan et al. 2011). FSP27 is notably in direct interaction with PLIN1a in white adipose tissues favoring the formation of LDs (Grahn et al. 2013; Sun et al. 2013). The size of LDs therefore seems regulated also by a balance between PLIN1a and FSP27. 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961

962 In plants, lipolysis three actors have been described: (1) a Sugar-Dependent
 963 Lipase type 1 (SDP1), (2) a homolog of the mammalian protein CGI-58 and (3) a
 964 peroxisomal transporter PXA1. These three actors are located at the level of the
 965 peroxisome upstream of lipolysis. SDP1 and its SDP1-like homolog (SDP1-L) have
 966 been found in the seed of *Arabidopsis thaliana* (Kelly et al. 2011). This enzyme is
 967 able to hydrolyze TAG, DAG, and MAG. SDP1 is a papatin-like lipase (Rydel et al.
 968 2003), homologous to mammalian ATGL, *S. cerevisiae* TAG lipases (TGL3, TGL4,
 969 TGL5) or *D. melanogaster* BRUMMER protein (Quettier and Eastmond 2009). A
 970 homolog of *Arabidopsis thaliana* SDP1 was also detected in the green alga
 971 *Lobosphaera incisa* (LiSDP1) (Siegler et al. 2017). A fusion with the fluorescent
 972 probe m-Venus showed the localization of this lipase at the surface of LDs of
 973 *L. incisa* (Siegler et al. 2017).

974 A loss of function of the CGI-58 protein in *Arabidopsis thaliana* results in an
 975 abnormal accumulation of LDs in the leaves (Ghosh et al. 2008; James et al. 2010).
 976 In addition, the seeds of plants deficient in CGI-58 contain equivalent amounts of
 977 neutral lipids, indicating that CGI-58 is not involved in the lipolysis mechanisms of
 978 the seed (James et al. 2010). Unlike mammals, the CGI-58 protein does not interact
 979 with ATGL lipase in plants but with an ATP-binding cassette (ABC) protein (PXA1)
 980 (Park et al. 2013). An ortholog of CGI-58 was found in the genome of the green
 981 microalga *Chlamydomonas reinhardtii* (Merchant et al. 2007). PXA1 (Peroxisomal
 982 ABC-Transporter 1) is localized at the level of the peroxisome and transports FAs
 983 upstream of their degradation by β -oxidation (Footitt et al. 2002). It has been
 984 estimated that 90% of FAs transported by PXA1 are provided by SDP1 in combi-
 985 nation with the retromer complex, a multiprotein complex involved in the
 986 recycling of transmembrane receptors and retrograde transport of cargo proteins
 987 from endosomes to *trans*-Golgi (Kelly and Feussner 2016). Physical contact
 988 between the LD and the peroxisome is negatively correlated with the presence of
 989 sucrose (Cui et al. 2016). PXA1 is also involved in the transfer of lipophilic pre-
 990 cursors for jasmonate signaling (Theodoulou et al. 2005) and CGI-58 in the regula-
 991 tion of polyamine metabolism (nitrogen metabolism), interacting with spermidine
 992 synthase 1 (SPDS1), in plants (Park et al. 2014).

993 Knowledge of the catabolism of LDs in secondary endosymbionts, containing
 994 secondary plastids, is very poor (Kong et al. 2018). A plant SDP1 lipase homolog,
 995 TGL1, was found in the diatom *Phaeodactylum* (Barka et al. 2016). A decrease in its
 996 expression causes the accumulation of TAGs. A second actor of catabolism of LDs
 997 was discovered in the diatom *Thalassiosira pseudonana*: CGI-58 (Trentacoste et al.
 998 2013). A decrease in the expression of CGI-58 in *Phaeodactylum* (antisense line)
 999 causes the accumulation of the amount of TAG (Leterrier et al. 2015). A recent
 1000 study, based on the search for conserved domains and phylogenetic analysis,
 1001 identified a putative TAG lipase in *Phaeodactylum*, called OmTGL (Li et al.
 1002 2018). By fusion with a fluorescent protein eGFP, this lipase was shown to localize
 1003 in the third outermost membrane of the chloroplast of *Phaeodactylum*. This locali-
 1004 zation is puzzling as this enzyme is supposed to operate on TAG originating from
 1005 cytosolic LDs. It seems that the dynamics of LDs in eukaryotes deriving from
 1006 secondary endosymbiosis have very complicated links with multiple membrane

compartments, including the two outermost membranes of the 4-membrane secondary plastid, the mitochondrial outer envelope membrane, peroxisomes, autophagosomes, etc. (Lupette et al. 2019).

Fatty Acid Degradation Via β -Oxidation

The β -oxidation pathway breaks down FAs and produces acetyl-CoA and reducing power (NADH and FADH₂) (Houten and Wanders 2010). This pathway occurs in the mitochondrial matrix in *Mammalia* and/or in the peroxisome (or glyoxysome) in photosynthetic organisms. Short chain fatty acids (less than eight carbons) are degraded in the mitochondria, whereas medium and very long chain fatty acids (greater than eight carbons) are degraded in the peroxisome (Dellero et al. 2018a). Activation and transport of FAs are two preliminary steps required prior β -oxidation initiation. FA activation is catalyzed by specific long chain acyl-CoA ligases. To reach the mitochondria, an acyl-CoA is ‘cargoes’ via the carnitine shuttle (Houten and Wanders 2010) in two steps: the acyl-CoA is first converted into acyl-carnitine at the outer mitochondrial membrane by carnitine palmitoyltransferase 1 (CPT1). Acylcarnitine then enters the mitochondrial intermembrane space by passing through a porin and then reaches the internal mitochondrial space via a carnitine-acylcarnitine translocase. To reach the peroxisome, transport of acyl-CoA occurs via a specific class of ABC transporter (ATP Binding Cassette).

In both mitochondria and peroxisomes, β -oxidation occurs by an interactive process, via the so-called Lynen helix (Houten and Wanders 2010).

11.4.3.3 Protein Control and Degradation by the ERAD Pathway and the 26S Proteasome

An ER localized pathway also regulates the proteins of cytosolic LDs in eukaryotic cells, called the ER associated protein degradation (ERAD) pathway. During translation, and following binding with the signal recognition protein (SRP), polypeptides carrying a signal peptide are imported by a translocon (Sec61 for example) into ER (Rapoport 2007), where they fold and undergo maturation, co- and post-translational modifications (e.g. cleavage of a signal peptide, *N*-glycosylation, and formation of disulfide bridges). The ERAD machinery is not only a system controlling the quality of proteins targeted to the ER but also a system controlling their quantity (Olzmann et al. 2013a; Stevenson et al. 2016). The presence of mutation, transcription, and translation errors may lead to misfolding, preventing a protein from reaching its functional conformation. The ERAD machinery operates in three stages. First, misfolded or mutated proteins are recognized in the ER. The presence of mismatched cysteine residues, immature glycans, or exposed hydrophobic regions may be targeted by chaperone proteins, such as in the latter case, chaperone proteins of the luminal binding protein (BIP) or cytosolic (Heat Shock Protein 70—Hsp70) (Okuda-Shimizu and Hendershot 2007). Misfolded proteins are then translocated from the

1046 ER to the cytosol. Translocation may involve a Valosin-containing-protein (VCP or
 1047 p97) in mammals or CDC48 (Cell Cycle Cycle 48 protein) in *Saccharomyces*
 1048 *cerevisiae* (Meyer et al. 2012). Finally, misfolded protein can be ubiquitinated and
 1049 degraded by the 26S proteasome (composed of a 20S barrel-shaped catalytic core in
 1050 the center and two 19S regulatory complexes at each end) (for review, see Sharma
 1051 et al. 2016). Multiple LD proteins are controlled by ERAD machinery via a process
 1052 that still needs to be fully elucidated (Ruggiano et al. 2016).

1053 ERAD machinery is also involved in the regulation of mammalian TAGs
 1054 (Stevenson et al. 2016). UBXD8 (UBX domain-containing protein 8) is an inhibitor
 1055 of TAG synthesis when upstream FAs synthesis is very low. An increase in FA
 1056 synthesis causes the delivery of a portion of UBDX8 from the ER to the LD, where it
 1057 recruits VCP (Valosin-containing protein) to the surface of the droplet (Suzuki et al.
 1058 2012; Olzmann et al. 2013b). This mechanism echoes the recruitment of CDC48
 1059 (another name of VCP) by PUX10 at the LD of the pollen tube of *Nicotiana tabacum*
 1060 (Kretzschmar et al. 2018). UBXD8 inhibits ATGL on the surface of the LD by
 1061 stimulating the dissociation of ATGL from its cofactor CGI-58 (Olzmann et al.
 1062 2013b).

1063 A second checkpoint occurs at the DGAT2 level. Treatment with PS-341, a
 1064 proteasome inhibitor, reduced the expression of DGAT2 (Oliva et al. 2012).
 1065 DGAT2 is a relatively unstable protein, degraded by the 26S proteasome after
 1066 ubiquitination (Choi et al. 2014). Gp78 (Glycoprotein 78) is an E3 ligase involved
 1067 in the ERAD mechanism (Chen et al. 2012). Functional analysis of this protein by
 1068 siRNA showed reduction in the ubiquitination of DGAT2 and an increase in its
 1069 stabilization (Choi et al. 2014). The transfer of the polyubiquitinated DGAT2 to the
 1070 proteasome is carried out by VCP. The role of UBDX8 in the degradation of DGAT2
 1071 is currently unknown (Stevenson et al. 2016).

1072 Overall, the ERAD machinery may be a conserved actor of LD homeostasis in
 1073 eukaryotes, but our knowledge is even more scarce compared to other regulatory
 1074 processes.

1075 11.5 Conclusion and Future Prospects

1076 This chapter shows that LDs and oil bodies are similar in their general organization
 1077 in prokaryotes and eukaryotes. Hydrophobic molecules loaded in their core show a
 1078 diversity of chemical structures (PHA, TAG, sterols, carotenoids, alkene, alkenone,
 1079 etc.). The PHA granules seem to be restricted to prokaryotes, whereas
 1080 TAG-containing LDs are found from bacteria to eukaryotes. The dynamics of LD
 1081 formation and homeostasis are exquisitely controlled, indicating the importance of
 1082 LDs in cell physiology and development. LDs are therefore essential for cell survival
 1083 and development, which is probably one of the reasons for the strong level of
 1084 conservation of these subcellular structures. Nevertheless, besides their evident
 1085 function as energy storage and carbon reservoirs, the variety of roles harbored by
 1086 LDs still needs to be investigated in major eukaryotic clades. It seems that in

eukaryotes, the ER plays a conserved role as a platform for LD formation, whereas mitochondria and peroxisome are critical for LD lipid mobilization. The association of proteins such as histones in LDs analyzed from insects to diatoms suggests that the LDs may have unexpected functions. LD biogenesis, dynamics, and role are nevertheless more complex in the organisms deriving from secondary endosymbiosis. Only a few LD-associated proteins seem to be conserved in eukaryotes, and some being involved in LD biogenesis (SEIPIN), TAG biosynthesis, TAG hydrolysis coupled with FA export from LDs (CGI-58). The evolution of CGI-58 is particularly puzzling since this component is involved in TAG mobilization from LDs, whereas CGI-58 molecular activity has apparently diverged between non-photosynthetic and photosynthetic clades. In photosynthetic organisms, the plastid seems to cooperate with the ER in LD biogenesis, possibly in relation to FA synthesis occurring in the stroma of this organelle, whereas FA synthesis occurs in the cytosol of non-photosynthetic eukaryotes. In eukaryotes containing secondary plastids, the role of this organelle may be even more important in LD biogenesis. A conclusion of this chapter may be a frustrating lack of knowledge, especially in important branches of the evolution of eukaryotes. Characterizing the molecular evolution of LD proteins and LD-controlling systems throughout the Tree of Life is a clear challenge for the future. This effort will be necessary to help explore biodiversity and identify oleaginous species, which may be novel promising resources for a multitude of applications from food, health to green chemistry and bioenergy.

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References

- Abe T, Kobayashi T, Saito T (2005) Properties of a novel intracellular poly(3-hydroxybutyrate) depolymerase with high specific activity (PhaZd) in *Wautersia eutropha* H16. *J Bacteriol* 187:6982–6990
- Acevedo F, Rubilar M, Shene C, Navarrete P, Romero F, Rabert C, Jolivet P, Valot B, Chardot T (2012) Seed oil bodies from *Gevuina avellana* and *Madia sativa*. *J Agric Food Chem* 60:6994–7004
- Aizaki H, Lee K-J, Sung VMH, Ishiko H, Lai MMC (2004) Characterization of the hepatitis C virus RNA replication complex associated with lipid rafts. *Virology* 324:450–461
- Alvarez HM (2016) Triacylglycerol and wax ester-accumulating machinery in prokaryotes. *Biochimie* 120:28–39
- Alvarez H, Steinbüchel A (2002) Triacylglycerols in prokaryotic microorganisms. *Appl Microbiol Biotechnol* 60:367–376
- Anderson AJ, Dawes EA (1990) Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. *Microbiol Rev* 54:450–472
- Archibald JM, Keeling PJ (2002) Recycled plastids: a ‘green movement’ in eukaryotic evolution. *Trends Genet* 18:577–584

- 1129 Arrese EL, Rivera L, Masakazu H, Mirza S, Hartson SD, Weintraub S, Soulages JL (2008) Function
1130 and structure of lipid storage droplet protein 1 studied in lipoprotein complexes. Arch Biochem
1131 Biophys 473:42–47
- 1132 Athenstaedt K, Zweytick D, Jandrositz A, Kohlwein SD, Daum G (1999) Identification and
1133 characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. J
1134 Bacteriol 181:6441–6448
- 1135 Athenstaedt K, Jolivet P, Boulard C, Zivy M, Negroni L, Nicaud J-M, Chardot T (2006) Lipid
1136 particle composition of the yeast *Yarrowia lipolytica* depends on the carbon source. Proteomics
1137 6:1450–1459
- 1138 Barka F, Angstenberger M, Ahrendt T, Lorenzen W, Bode HB, Büchel C (2016) Identification of a
1139 triacylglycerol lipase in the diatom *Phaeodactylum tricornutum*. Biochim Biophys Acta
1140 1861:239–248
- 1141 Bartz R, Li W-H, Venables B, Zehmer JK, Roth MR, Welti R, Anderson RGW, Liu P, Chapman
1142 KD (2007) Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid
1143 traffic. J Lipid Res 48:837–847
- 1144 Baud S, Dichow NR, Kelemen Z, D'andréa S, To A, Berger N, Canonge M, Kronenberger J,
1145 Viterbo D, Dubreucq B, Lepiniec L, Chardot T, Miquel M (2009) Regulation of HSD1 in seeds
1146 of *Arabidopsis thaliana*. Plant Cell Physiol 50:1463–1478
- 1147 Beck R, Ravet M, Wieland FT, Cassel D (2009) The COPI system: molecular mechanisms and
1148 function. FEBS Lett 583:2701–2709
- 1149 Beller M, Riedel D, Jänsch L, Dieterich G, Wehland J, Jäckle H, Kühnlein RP (2006) Character-
1150 ization of the *Drosophila* lipid droplet subproteome. Mol Cell Proteomics 5:1082–1094
- 1151 Beller M, Sztalryd C, Southall N, Bell M, Jäckle H, Auld DS, Oliver B (2008) COPI complex is a
1152 regulator of lipid homeostasis. PLoS Biol 6:e292
- 1153 Beller M, Bulankina AV, Hsiao H-H, Urlaub H, Jäckle H, Kühnlein RP (2010) PERILIPIN-
1154 dependent control of lipid droplet structure and fat storage in drosophila. Cell Metab
1155 12:521–532
- 1156 Bendif EM, Probert I, Schroeder DC, De Vargas C (2013) On the description of *Tisochrysis lutea*
1157 gen. nov. sp. nov. and *Isochrysis nuda* sp. nov. in the Isochrysidales, and the transfer of
1158 Dicrateria to the Prymnesiales (Haptophyta). J Appl Phycol 25:1763–1776
- 1159 Bersuker K, Olzmann JA (2017) Establishing the lipid droplet proteome: mechanisms of lipid
1160 droplet protein targeting and degradation. Biochim Biophys Acta 1862:1166–1177
- 1161 Berthelot K, Lecomte S, Estevez Y, Zhendre V, Henry S, Thévenot J, Dufourc EJ, Alves ID, Peruch
1162 F (2014) Rubber particle proteins, HbREF and HbSRPP, show different interactions with model
1163 membranes. Biochim Biophys Acta Biomembr 1838:287–299
- 1164 Bi J, Xiang Y, Chen H, Liu Z, Grönke S, Kühnlein RP, Huang X (2012) Opposite and redundant
1165 roles of the two *Drosophila* perilipins in lipid mobilization. J Cell Sci 125:3568
- 1166 Bickel PE, Tansey JT, Welte MA (2009) PAT proteins, an ancient family of lipid droplet proteins
1167 that regulate cellular lipid stores. Biochim Biophys Acta 1791:419–440
- 1168 Boyer A, Dumans A, Beaumont E, Etienne L, Roingeard P, Meunier J-C (2014) The association of
1169 hepatitis C virus glycoproteins with apolipoproteins E and B early in assembly is conserved in
1170 lipoviral particles. J Biol Chem 289:18904–18913
- 1171 Brasaemle DL, Wolins NE (2016) Isolation of lipid droplets from cells by density gradient
1172 centrifugation. Curr Protoc Cell Biol 72:3.15.11–13.15.13
- 1173 Bréhélin C, Kessler F, Van Wijk KJ (2007) Plastoglobules: versatile lipoprotein particles in
1174 plastids. Trends Plant Sci 12:260–266
- 1175 Bresan S, Sznajder A, Hauf W, Forchhammer K, Pfeiffer D, Jendrosseck D (2016)
1176 Polyhydroxyalkanoate (PHA) granules have no phospholipids. Sci Rep 6:26612
- 1177 Brocard L, Immel F, Coulon D, Esnay N, Tuphile K, Pascal S, Claverol S, Fouille L, Bessoule J-J,
1178 Bréhélin C (2017) Proteomic analysis of lipid droplets from *Arabidopsis* aging leaves brings
1179 new insight into their biogenesis and functions. Front Plant Sci 8:894
- 1180 Bussell R, Eliezer D (2003) A structural and functional role for 11-mer repeats in α -synuclein and
1181 other exchangeable lipid binding proteins. J Mol Biol 329:763–778

- Cai Y, Goodman JM, Pyc M, Mullen RT, Dyer JM, Chapman KD (2015) Arabidopsis SEIPIN proteins modulate triacylglycerol accumulation and influence lipid droplet proliferation. *Plant Cell* 27:2616–2636 1182–1184
- Caire-Brändli I, Papadopoulos A, Malaga W, Marais D, Canaan S, Thilo L, De Chastellier C (2014) Reversible lipid accumulation and associated division arrest of mycobacterium avium lipoprotein-induced foamy macrophages may resemble key events during latency and reactivation of tuberculosis. *Infect Immun* 82:476–490 1186–1188
- Camus G, Herker E, Modi AA, Haas JT, Ramage HR, Farese RV, Ott M (2013) Diacylglycerol acyltransferase-1 localizes hepatitis C virus NS5A protein to lipid droplets and enhances NS5A interaction with the viral capsid core. *J Biol Chem* 288:9915–9923 1189–1191
- Castorena KM, Stapleford KA, Miller DJ (2010) Complementary transcriptomic, lipidomic, and targeted functional genetic analyses in cultured *Drosophila* cells highlight the role of glycerophospholipid metabolism in Flock House virus RNA replication. *BMC Genomics* 11:183–183 1192–1195
- Cavalier-Smith T (1986) The kingdom Chromista: origin and systematics. Biopress, Bristol 1196
- Cavalier-Smith T (2018) Kingdom Chromista and its eight phyla: a new synthesis emphasising periplastid protein targeting, cytoskeletal and periplastid evolution, and ancient divergences. *Protoplasma* 255:297–357 1197–1199
- Cermelli S, Guo Y, Gross SP, Welte MA (2006) The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Curr Biol* 16:1783–1795 1200–1201
- Chang BH-J, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, Chan L (2006) Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein. *Mol Cell Biol* 26:1063–1076 1202–1204
- Chapman KD, Dyer JM, Mullen RT (2012) Biogenesis and functions of lipid droplets in plants: thematic review series: lipid droplet synthesis and metabolism: from yeast to man. *J Lipid Res* 53:215–226 1205–1207
- Chen JC, Tsai CC, Tzen JT (1999) Cloning and secondary structure analysis of caleosin, a unique calcium-binding protein in oil bodies of plant seeds. *Plant Cell Physiol* 40:1079–1086 1208–1209
- Chen Z, Du S, Fang S (2012) gp78: a multifaceted ubiquitin ligase that integrates a unique protein degradation pathway from the endoplasmic reticulum. *Curr Protein Pept Sci* 13:414–424 1210–1211
- Chen W, Chang B, Wu X, Li L, Sleeman M, Chan L (2013) Inactivation of Plin4 downregulates Plin5 and reduces cardiac lipid accumulation in mice. *Am J Physiol-Endocrinol Metab* 304:E770–E779 1212–1214
- Chen Y, Ding Y, Yang L, Yu J, Liu G, Wang X, Zhang S, Yu D, Song L, Zhang H, Zhang C, Huo L, Huo C, Wang Y, Du Y, Zhang H, Zhang P, Na H, Xu S, Zhu Y, Xie Z, He T, Zhang Y, Wang G, Fan Z, Yang F, Liu H, Wang X, Zhang X, Zhang MQ, Li Y, Steinbüchel A, Fujimoto T, Cichello S, Yu J, Liu P (2014) Integrated omics study delineates the dynamics of lipid droplets in *Rhodococcus opacus* PD630. *Nucleic Acids Res* 42:1052–1064 1215–1219
- Choi K, Kim H, Kang H, Lee S-Y, Lee SJ, Back SH, Lee SH, Kim MS, Lee JE, Park JY, Kim J, Kim S, Song J-H, Choi Y, Lee S, Lee H-J, Kim JH, Cho S (2014) Regulation of diacylglycerol acyltransferase 2 protein stability by gp78-associated endoplasmic-reticulum-associated degradation. *FEBS J* 281:3048–3060 1220–1223
- Choudhary V, Golden A, Prinz WA (2016) Keeping FIT, storing fat: lipid droplet biogenesis. *Worm* 5:e1170276 1224–1225
- Chughtai AA, Kaššák F, Kostrouchová M, Novotný JP, Krause MW, Saudek V, Kostrouch Z, Kostrouchová M (2015) Perilipin-related protein regulates lipid metabolism in *C. elegans*. *PeerJ* 3:e1213 1226–1228
- Chung J, Wu X, Lambert TJ, Lai ZW, Walther TC, Farese RV (2019) LDAF1 and seipin form a lipid droplet assembly complex. *Dev Cell* 51:551–563.e557 1229–1230
- Cui S, Hayashi Y, Otomo M, Mano S, Oikawa K, Hayashi M, Nishimura M (2016) Sucrose production mediated by lipid metabolism suppresses physical interaction of peroxisomes and oil bodies during germination of *Arabidopsis thaliana*. *J Biol Chem* 291:19734–19745 1231–1233

- 1234 Czabany T, Wagner A, Zweytick D, Lohner K, Leitner E, Ingolic E, Daum G (2008) Structural and
 1235 biochemical properties of lipid particles from the yeast *Saccharomyces cerevisiae*. J Biol Chem
 1236 283:17065–17074
- 1237 D'avila H, Freire-De-Lima CG, Roque NR, Teixeira L, Barja-Fidalgo C, Silva AR, Melo RCN,
 1238 Dosreis GA, Castro-Faria-Neto HC, Bozza PT (2011) Host cell lipid bodies triggered by
 1239 *Trypanosoma cruzi* infection and enhanced by the uptake of apoptotic cells are associated
 1240 with prostaglandin E2 generation and increased parasite growth. J Infect Dis 204:951–961
- 1241 D'andrea S (2016) Lipid droplet mobilization: the different ways to loosen the purse strings.
 1242 Biochimie 120:17–27
- 1243 Davidi L, Katz A, Pick U (2012) Characterization of major lipid droplet proteins from *Dunaliella*.
 1244 Planta 236:19–33
- 1245 Davidi L, Levin Y, Ben-Dor S, Pick U (2015) Proteome analysis of cytoplasmatic and plastidic β -
 1246 carotene lipid droplets in *Dunaliella bardawil*. Plant Physiol 167:60–79
- 1247 Dellerio Y, Cagnac O, Rose S, Seddiki K, Cussac M, Morabito C, Lupette J, Aiese Cigliano R,
 1248 Sanseverino W, Kuntz M, Jouhet J, Maréchal E, Rébeillé F, Amato A (2018a) Proposal of a new
 1249 thraustochytrid genus *Hondaea* gen. nov. and comparison of its lipid dynamics with the closely
 1250 related pseudo-cryptic genus *Aurantiochytrium*. Algal Res 35:125–141
- 1251 Dellerio Y, Rose S, Metton C, Morabito C, Lupette J, Jouhet J, Maréchal E, Rébeillé F, Amato A
 1252 (2018b) Ecophysiology and lipid dynamics of a eukaryotic mangrove decomposer. Environ
 1253 Microbiol 20:3057–3068
- 1254 Den Brok MH, Raaijmakers TK, Collado-Camps E, Adema GJ (2018) Lipid droplets as immune
 1255 modulators in myeloid cells. Trends Immunol 39:380–392
- 1256 Dias-Lopes G, Borges-Veloso A, Saboia-Vahia L, Padrón G, De Faria Castro CL, Guimarães ACR,
 1257 Britto C, Cuervo P, De Jesus JB (2016) Proteomics reveals major components of oogenesis in
 1258 the reproductive tract of sugar-fed *Anopheles aquasalis*. Parasitol Res 115:1977–1989
- 1259 Ding Y, Wu Y, Zeng R, Liao K (2012a) Proteomic profiling of lipid droplet-associated proteins in
 1260 primary adipocytes of normal and obese mouse. Acta Biochim Biophys Sin 44:394–406
- 1261 Ding Y, Yang L, Zhang S, Wang Y, Du Y, Pu J, Peng G, Chen Y, Zhang H, Yu J, Hang H, Wu P,
 1262 Yang F, Yang H, Steinbüchel A, Liu P (2012b) Identification of the major functional proteins of
 1263 prokaryotic lipid droplets. J Lipid Res 53:399–411
- 1264 Du X, Barisch C, Paschke P, Herrfurth C, Bertinetti O, Pawolleck N, Otto H, Rühling H, Feussner I,
 1265 Herberg FW, Maniak M (2013) Dictyostelium lipid droplets host novel proteins. Eukaryot Cell
 1266 12:1517–1529
- 1267 Eggers J, Steinbüchel A (2013) Poly(3-hydroxybutyrate) degradation in *Ralstonia eutropha* H16 is
 1268 mediated stereoselectively to (S)-3-hydroxybutyryl coenzyme a (CoA) via crotonyl-CoA. J
 1269 Bacteriol 195:3213–3223
- 1270 Faucher P, Poitou C (2016) Physiopathologie de l'obésité. Rev Rhum Monogr 83:6–12
- 1271 Fei W, Shui G, Gaeta B, Du X, Kuerschner L, Li P, Brown AJ, Wenk MR, Parton RG, Yang H
 1272 (2008) Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in
 1273 yeast. J Cell Biol 180:473–482
- 1274 Footitt S, Slocombe SP, Larner V, Kurup S, Wu Y, Larson T, Graham I, Baker A, Holdsworth M
 1275 (2002) Control of germination and lipid mobilization by COMATOSE, the Arabidopsis homo-
 1276 logue of human ALDP. EMBO J 21:2912–2922
- 1277 Frandsen G, Müller-Uri F, Nielsen M, Mundy J, Skriver K (1996) Novel plant Ca-binding protein
 1278 expressed in response to abscisic acid and osmotic stress. J Biol Chem 271:343–348
- 1279 Füßy Z, Oborník M (2018) Complex endosymbioses I: from primary to complex plastids, multiple
 1280 independent events. In: Maréchal E (ed) Plastids: methods and protocols. Springer, New York,
 1281 pp 17–35
- 1282 Gaunt ER, Zhang Q, Cheung W, Wakelam MJO, Lever AML, Desselberger U (2013) Lipidome
 1283 analysis of rotavirus-infected cells confirms the close interaction of lipid droplets with
 1284 viroplasms. J Gen Virol 94:1576–1586
- 1285 Georgieva D, Schmitt V, Leal-Calderon F, Langevin D (2009) On the possible role of surface
 1286 elasticity in emulsion stability. Langmuir 25:5565–5573

- Gerngross TU, Reilly P, Stubbe J, Sinskey AJ, Peoples OP (1993) Immunocytochemical analysis of poly-beta-hydroxybutyrate (PHB) synthase in *Alcaligenes eutrophus* H16: localization of the synthase enzyme at the surface of PHB granules. *J Bacteriol* 175:5289–5293
- Ghosh AK, Ramakrishnan G, Chandramohan C, Rajasekharan R (2008) CGI-58, the causative gene for Chanarin-Dorfman syndrome, mediates acylation of lysophosphatidic acid. *J Biol Chem* 283:24525–24533
- Gidda SK, Park S, Pyc M, Yurchenko O, Cai Y, Wu P, Andrews DW, Chapman KD, Dyer JM, Mullen RT (2016) Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells. *Plant Physiol* 170:2052–2071
- Gocze PM, Freeman DA (1994) Factors underlying the variability of lipid droplet fluorescence in MA-10 leydig tumor cells. *Cytometry* 17:151–158
- Gong J, Sun Z, Wu L, Xu W, Schieber N, Xu D, Shui G, Yang H, Parton RG, Li P (2011) Fsp27 promotes lipid droplet growth by lipid exchange and transfer at lipid droplet contact sites. *J Cell Biol* 195:953–963
- Goold H, Beisson F, Peltier G, Li-Beisson Y (2015) Microalgal lipid droplets: composition, diversity, biogenesis and functions. *Plant Cell Rep* 34:545–555
- Grahn THM, Zhang Y, Lee M-J, Sommer AG, Mostoslavsky G, Fried SK, Greenberg AS, Puri V (2013) FSP27 and PLIN1 interaction promotes the formation of large lipid droplets in human adipocytes. *Biochem Biophys Res Commun* 432:296–301
- Granneman JG, Moore H-PH, Krishnamoorthy R, Rathod M (2009) Perilipin controls lipolysis by regulating the interactions of AB-hydrolase containing 5 (Abhd5) and adipose triglyceride lipase (Atgl). *J Biol Chem* 284:34538–34544
- Granneman JG, Kimler VA, Zhang H, Ye X, Luo X, Postlethwait JH, Thummel R (2017) Lipid droplet biology and evolution illuminated by the characterization of a novel perilipin in teleost fish. *elife* 6:e21771
- Greenspan P, Mayer EP, Fowler SD (1985) Nile red: a selective fluorescent stain for intracellular lipid droplets. *J Cell Biol* 100:965–973
- Grillitsch K, Connerth M, Köfeler H, Arrey TN, Rietschel B, Wagner B, Karas M, Daum G (2011) Lipid particles/droplets of the yeast *Saccharomyces cerevisiae* revisited: lipidome meets proteome. *Biochim Biophys Acta* 1811:1165–1176
- Gromova M, Guillermo A, Bayle P-A, Bardet M (2015) In vivo measurement of the size of oil bodies in plant seeds using a simple and robust pulsed field gradient NMR method. *Eur Biophys J* 44:121–129
- Guo Y, Walther TC, Rao M, Stuurman N, Goshima G, Terayama K, Wong JS, Vale RD, Walter P, Farese RV (2008) Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* 453:657–661
- Guo B, Lei C, Ito T, Yaxiaer Y, Kobayashi H, Jiang Y, Tanaka Y, Ozeki Y, Goda K (2017) High-throughput label-free screening of *euglena gracilis* with optofluidic time-stretch quantitative phase microscopy. *SPIE BiOS* 12
- Hagel JM, Yeung EC, Facchini PJ (2008) Got milk? The secret life of laticifers. *Trends Plant Sci* 13:631–639
- Hanano A, Burcklen M, Flenet M, Ivancich A, Louwagie M, Garin J, Blée E (2006) Plant seed peroxxygenase is an original heme-oxygenase with an EF-hand calcium binding motif. *J Biol Chem* 281:33140–33151
- Harchouni S, Field B, Menand B (2018) AC-202, a highly effective fluorophore for the visualization of lipid droplets in green algae and diatoms. *Biotechnol Biofuels* 11:120
- Haywood GW, Anderson AJ, Dawes EA (1989) The importance of PHB-synthase substrate specificity in polyhydroxyalkanoate synthesis by *Alcaligenes eutrophus*. *FEMS Microbiol Lett* 57:1–6
- Hehlert P, Hofferek V, Heier C, Eichmann TO, Riedel D, Rosenberg J, Takaács A, Nagy HM, Oberer M, Zimmermann R, Kühnlein RP (2019) The α/β -hydrolase domain-containing 4- and 5-related phospholipase Pummelig controls energy storage in *Drosophila*. *J Lipid Res* 60:1365–1378

- 1340 Henne WM, Reese ML, Goodman JM (2018) The assembly of lipid droplets and their roles in
 1341 challenged cells. *EMBO J* 37
- 1342 Horn PJ, James CN, Gidda SK, Kilaru A, Dyer JM, Mullen RT, Ohlrogge JB, Chapman KD (2013)
 1343 Identification of a new class of lipid droplet-associated proteins in plants. *Plant Physiol*
 1344 162:1926–1936
- 1345 Houten SM, Wanders RJA (2010) A general introduction to the biochemistry of mitochondrial fatty
 1346 acid β -oxidation. *J Inher Metab Dis* 33:469–477
- 1347 Hsieh K, Huang AHC (2004) Endoplasmic reticulum, oleosins, and oils in seeds and tapetum cells.
 1348 *Plant Physiol* 136:3427–3434
- 1349 Hu X, Binns D, Reese ML (2017) The coccidian parasites *Toxoplasma* and *Neospora* dysregulate
 1350 mammalian lipid droplet biogenesis. *J Biol Chem* 292:11009–11020
- 1351 Huang AH (1996) Oleosins and oil bodies in seeds and other organs. *Plant Physiol* 110:1055–1061
- 1352 Huang AHC (2018) Plant lipid droplets and their associated proteins: potential for rapid advances
 1353 ([OPEN]). *Plant Physiol* 176:1894–1918
- 1354 Huang M-D, Huang AHC (2015) Bioinformatics reveal five lineages of oleosins and the mechanism
 1355 of lineage evolution related to structure/function from green algae to seed plants. *Plant Physiol*
 1356 169:453–470
- 1357 Hubert F, Poisson L, Loiseau C, Gauvry L, Pencreac'h G, Hérault J, Ergon F (2017) Lipids and
 1358 lipolytic enzymes of the microalga *Isochrysis galbana*. *OCL* 24:D407
- 1359 Ikari N, Shimizu A, Asano T (2018) Lysosomal acid lipase deficiency in Japan: a case report of
 1360 siblings and a literature review of cases in Japan. *J Nippon Med Sch* 85:131–137
- 1361 Imai Y, Varela GM, Jackson MB, Graham MJ, Crooke RM, Ahima RS (2007) Reduction of
 1362 hepatosteatosis and lipid levels by an adipose differentiation-related protein antisense oligonu-
 1363 cleotide. *Gastroenterology* 132:1947–1954
- 1364 Imamura M, Inoguchi T, Ikuyama S, Taniguchi S, Kobayashi K, Nakashima N, Nawata H (2002)
 1365 ADRP stimulates lipid accumulation and lipid droplet formation in murine fibroblasts. *Am J*
 1366 *Physiol-Endocrinol Metab* 283:E775–E783
- 1367 Ivashov VA, Grillitsch K, Koefeler H, Leitner E, Baumlisberger D, Karas M, Daum G (2013)
 1368 Lipidome and proteome of lipid droplets from the methylotrophic yeast *Pichia pastoris*.
 1369 *Biochim Biophys Acta* 1831:282–290
- 1370 Jackson LP (2014) Structure and mechanism of COPI vesicle biogenesis. *Curr Opin Cell Biol*
 1371 29:67–73
- 1372 Jambunathan S, Yin J, Khan W, Tamori Y, Puri V (2011) FSP27 promotes lipid droplet clustering
 1373 and then fusion to regulate triglyceride accumulation. *PLoS One* 6:e28614
- 1374 James CN, Horn PJ, Case CR, Gidda SK, Zhang D, Mullen RT, Dyer JM, Anderson RGW,
 1375 Chapman KD (2010) Disruption of the Arabidopsis CGI-58 homologue produces Chananin-
 1376 Dorfman-like lipid droplet accumulation in plants. *Proc Natl Acad Sci USA* 107:17833–17838
- 1377 James GO, Hocart CH, Hillier W, Chen H, Kordabach F, Price GD, Djordjevic MA (2011) Fatty
 1378 acid profiling of *Chlamydomonas reinhardtii* under nitrogen deprivation. *Bioresour Technol*
 1379 102:3343–3351
- 1380 Javee A, Sulochana SB, Pallissery SJ, Arumugam M (2016) Major lipid body protein: a conserved
 1381 structural component of lipid body accumulated during abiotic stress in *S. quadricauda* CASA-
 1382 CC202. *Front Energy Res* 4
- 1383 Jiang P-L, Pasariu B, Chen C-S (2014) Nitrogen-deprivation elevates lipid levels in
 1384 Symbiodinium spp. by lipid droplet accumulation: morphological and compositional analyses.
 1385 *PLoS One* 9:e87416
- 1386 Johnson MR, Stephenson RA, Ghaemmaghami S, Welte MA (2018) Developmentally regulated
 1387 H2Av buffering via dynamic sequestration to lipid droplets in Drosophila embryos. *elife* 7:
 1388 e36021
- 1389 Jolivet P, Roux E, D'andrea S, Davanture M, Negroni L, Zivy M, Chardot T (2004) Protein
 1390 composition of oil bodies in *Arabidopsis thaliana* ecotype WS. *Plant Physiol Biochem*
 1391 42:501–509

- Jolivet P, Boulard C, Bellamy A, Larré C, Barre M, Rogniaux H, D'andréa S, Chardot T, Nesi N (2009) Protein composition of oil bodies from mature *Brassica napus* seeds. *Proteomics* 9:3268–3284
- Jolivet P, Acevedo F, Boulard C, D'andréa S, Faure J-D, Kohli A, Nesi N, Valot B, Chardot T (2013) Crop seed oil bodies: from challenges in protein identification to an emerging picture of the oil body proteome. *Proteomics* 13:1836–1849
- Kalscheuer R, Wältermann M, Alvarez H, Steinbüchel A (2001) Preparative isolation of lipid inclusions from *Rhodococcus opacus* and *Rhodococcus ruber* and identification of granule-associated proteins. *Arch Microbiol* 177:20–28
- Kalscheuer R, Stöveken T, Malkus U, Reichelt R, Golyshin PN, Sabirova JS, Ferrer M, Timmis KN, Steinbüchel A (2007) Analysis of storage lipid accumulation in *Alcanivorax borkumensis*: evidence for alternative triacylglycerol biosynthesis routes in bacteria. *J Bacteriol* 189:918–928
- Katavic V, Agrawal GK, Hajduch M, Harris SL, Thelen JJ (2006) Protein and lipid composition analysis of oil bodies from two *Brassica napus* cultivars. *Proteomics* 6:4586–4598
- Kellogg RB, Patton JS (1983) Lipid droplets, medium of energy exchange in the symbiotic anemone *Condylactis gigantea*: a model coral polyp. *Mar Biol* 75:137–149
- Kelly AA, Feussner I (2016) Oil is on the agenda: lipid turnover in higher plants. *Biochim Biophys Acta* 1861:1253–1268
- Kelly AA, Quettier A-L, Shaw E, Eastmond PJ (2011) Seed storage oil mobilization is important but not essential for germination or seedling establishment in *Arabidopsis*. *Plant Physiol* 157:866–875
- Khandelia H, Duelund L, Pakkanen KI, Ipsen JH (2010) Triglyceride blisters in lipid bilayers: implications for lipid droplet biogenesis and the mobile lipid signal in cancer cell membranes. *PLoS One* 5:e12811
- Kilaru A, Cao X, Dabbs PB, Sung H-J, Rahman MM, Thrower N, Zynda G, Podicheti R, Ibarra-Laclette E, Herrera-Estrella L, Mockaitis K, Ohlrogge JB (2015) Oil biosynthesis in a basal angiosperm: transcriptome analysis of *Persea americana* mesocarp. *BMC Plant Biol* 15:203–203
- Kim M-J, Wainwright HC, Locketz M, Bekker L-G, Walther GB, Dittrich C, Visser A, Wang W, Hsu F-F, Wiehart U, Tsenova L, Kaplan G, Russell DG (2010) Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. *EMBO Mol Med* 2:258–274
- Kimmel AR, Sztalryd C (2014) Perilipin 5, a lipid droplet protein adapted to mitochondrial energy utilization. *Curr Opin Lipidol* 25:110–117
- Kimmel AR, Sztalryd C (2016) The perilipins: major cytosolic lipid droplet-associated proteins and their roles in cellular lipid storage, mobilization, and systemic homeostasis. *Annu Rev Nutr* 36:471–509
- Kimmel AR, Brasaemle DL, Mcandrews-Hill M, Sztalryd C, Londos C (2010) Adoption of PERILIPIN as a unifying nomenclature for the mammalian PAT-family of intracellular lipid storage droplet proteins. *J Lipid Res* 51:468–471
- Koch B, Schmidt C, Daum G (2014) Storage lipids of yeasts: a survey of nonpolar lipid metabolism in *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Yarrowia lipolytica*. *FEMS Microbiol Rev* 38:892–915
- Kong F, Romero IT, Warakanont J, Li-Beisson Y (2018) Lipid catabolism in microalgae. *New Phytol* 218:1340–1348
- Kory N, Farese RV, Walther TC (2016) Targeting fat: mechanisms of protein localization to lipid droplets. *Trends Cell Biol* 26:535–546
- Kreimer G (2009) The green algal eyespot apparatus: a primordial visual system and more? *Curr Genet* 55:19–43
- Kretzschmar FK, Mengel LF, Müller A, Schmitt K, Bliersch KF, Valerius O, Braus G, Ischebeck T (2018) PUX10 is a lipid droplet-localized scaffold protein that interacts with CDC48 and is involved in the degradation of lipid droplet proteins. *Plant Cell* 30:2137–2160
- Kuerschner L, Moessinger C, Thiele C (2007) Imaging of lipid biosynthesis: how a neutral lipid enters lipid droplets. *Traffic* 9:338–352

- 1445 Kumar Y, Cocchiari J, Valdivia RH (2006) The obligate intracellular pathogen *Chlamydia*
 1446 *trachomatis* targets host lipid droplets. *Curr Biol* 16:1646–1651
- 1447 Kuntam S, Puskás LG, Ayaydin F (2015) Characterization of a new class of blue-fluorescent lipid
 1448 droplet markers for live-cell imaging in plants. *Plant Cell Rep* 34:655–665
- 1449 Laibach N, Post J, Twyman RM, Gronover CS, Prüfer D (2015) The characteristics and potential
 1450 applications of structural lipid droplet proteins in plants. *J Biotechnol* 201:15–27
- 1451 Leber R, Zinser E, Paltauf F, Daum G, Zellnig G (1994) Characterization of lipid particles of the
 1452 yeast, *Saccharomyces cerevisiae*. *Yeast* 10:1421–1428
- 1453 Lee JH, Kong J, Jang JY, Han JS, Ji Y, Lee J, Kim JB (2014) Lipid droplet protein LID-1 mediates
 1454 ATGL-1-dependent lipolysis during fasting in *Caenorhabditis elegans*. *Mol Cell Biol*
 1455 34:4165–4176
- 1456 Lee YK, Sohn JH, Han JS, Park YJ, Jeon YG, Ji Y, Dalen KT, Sztalryd C, Kimmel AR, Kim JB
 1457 (2018) *Perilipin 3* deficiency stimulates thermogenic beige adipocytes through *PPARα* activa-
 1458 tion. *Diabetes* 67:791–804
- 1459 Lersten NR, Czapinski AR, Curtis JD, Freckmann R, Horner HT (2006) Oil bodies in leaf
 1460 mesophyll cells of angiosperms: overview and a selected survey. *Am J Bot* 93:1731–1739
- 1461 Leterrier M, Calleja P, Maréchal E (2015) Modified algae strain and method of triacylglycerol
 1462 accumulation using said strain
- 1463 Li Z, Thiel K, Thul PJ, Beller M, Kühnlein RP, Welte MA (2012) Lipid droplets control the
 1464 maternal histone supply of *Drosophila* embryos. *Curr Biol* 22:2104–2113
- 1465 Li X, Pan Y, Hu H (2018) Identification of the triacylglycerol lipase in the chloroplast envelope of
 1466 the diatom *Phaeodactylum tricornutum*. *Algal Res* 33:440–447
- 1467 Lin L-J, Liao P-C, Yang H-H, Tzen JTC (2005) Determination and analyses of the N-termini of
 1468 oil-body proteins, steroleosin, caleosin and oleosin. *Plant Physiol Biochem* 43:770–776
- 1469 Lin IP, Jiang P-L, Chen C-S, Tzen JTC (2012) A unique caleosin serving as the major integral
 1470 protein in oil bodies isolated from *Chlorella* sp. cells cultured with limited nitrogen. *Plant*
 1471 *Physiol Biochem* 61:80–87
- 1472 Lin P, Chen X, Moktan H, Arrese EL, Duan L, Wang L, Soulages JL, Zhou DH (2014) Membrane
 1473 attachment and structure models of lipid storage droplet protein 1. *Biochim Biophys Acta*
 1474 1838:874–881
- 1475 Liu H, Wang C, Chen F, Shen S (2015) Proteomic analysis of oil bodies in mature *Jatropha curcas*
 1476 seeds with different lipid content. *J Proteome* 113:403–414
- 1477 Low KL, Shui G, Natter K, Yeo WK, Kohlwein SD, Dick T, Rao SPS, Wenk MR (2010) Lipid
 1478 droplet-associated proteins are involved in the biosynthesis and hydrolysis of triacylglycerol in
 1479 *Mycobacterium bovis* bacillus Calmette-Guérin. *J Biol Chem* 285:21662–21670
- 1480 Lu Y, Wang X, Balamurugan S, Yang W-D, Liu J-S, Dong H-P, Li H-Y (2017) Identification of a
 1481 putative seipin ortholog involved in lipid accumulation in marine microalga *Phaeodactylum*
 1482 *tricornutum*. *J Appl Phycol* 29:2821–2829
- 1483 Lundin C, Nordström R, Wagner K, Windpassinger C, Andersson H, Von Heijne G, Nilsson I
 1484 (2006) Membrane topology of the human seipin protein. *FEBS Lett* 580:2281–2284
- 1485 Luo M, Fadeev EA, Groves JT (2005) Mycobactin-mediated iron acquisition within macrophages.
 1486 *Nat Chem Biol* 1:149
- 1487 Lupette J, Maréchal E (2018) Phytoplankton glycerolipids: challenging but promising prospects
 1488 from biomedicine to green chemistry and biofuels. *Blue Biotechnology*
- 1489 Lupette J, Lami R, Krasovec M, Grimsley N, Moreau H, Piganeau G, Sanchez-Ferandin S (2016)
 1490 *Marinobacter* dominates the bacterial community of the *Ostreococcus tauri* phycosphere in
 1491 culture. *Front Microbiol* 7:1414
- 1492 Lupette J, Jaussaud A, Seddiki K, Morabito C, Brugière S, Schaller H, Kuntz M, Putaux J-L,
 1493 Jouneau P-H, Rébeillé F, Falconet D, Couté Y, Jouhet J, Tardif M, Salvaing J, Maréchal E
 1494 (2019) The architecture of lipid droplets in the diatom *Phaeodactylum tricornutum*. *Algal Res*
 1495 38:101415
- 1496 Madrigal-Matute J, Cuervo AM (2016) Regulation of liver metabolism by autophagy. *Gastroen-
 1497 terology* 150:328–339

- Maeda Y, Sunaga Y, Yoshino T, Tanaka T (2014) Oleosome-associated protein of the oleaginous diatom *Fistulifera solaris* contains an endoplasmic reticulum-targeting signal sequence. *Mar Drugs* 12:3892–3903 1498 1500
- Magré J, Delépine M, Khallouf E, Gedde-Dahl T Jr, Van Maldergem L, Sobel E, Papp J, Meier M, Mégarbané A, BWG, Lathrop M, Capeau J (2001) Identification of the gene altered in Berardinelli–Seip congenital lipodystrophy on chromosome 11q13. *Nat Genet* 28:365 1502 1503
- Malchow D, Lüderitz O, Westphal O, Gerisch G, Riedel V (1967) Polysaccharide in vegetativen und aggregationsreifen Amöben von *Dictyostelium discoideum*. *Eur J Biochem* 2:469–479 1504 1505
- Maréchal E (2018) Primary endosymbiosis: emergence of the primary chloroplast and the chromatinophore, two independent events. In: Maréchal E (ed) *Plastids: methods and protocols*. Springer, New York, pp 3–16 1506 1507 1508
- Marlowe IT, Brassell SC, Eglinton G, Green JC (1984a) Long chain unsaturated ketones and esters in living algae and marine sediments. *Org Geochem* 6:135–141 1509 1510
- Marlowe IT, Green JC, Neal AC, Brassell SC, Eglinton G, Course PA (1984b) Long chain (n-C37–C39) alkenones in the Prymnesiophyceae. Distribution of alkenones and other lipids and their taxonomic significance. *Br Phycol J* 19:203–216 1511 1512 1513
- Mayer SV, Tesh RB, Vasilakis N (2017) The emergence of arthropod-borne viral diseases: a global prospective on dengue, chikungunya and zika fevers. *Acta Trop* 166:155–163 1514 1515
- Mcmanaman JL, Bales ES, Orlicky DJ, Jackman M, Maclean PS, Cain S, Crunk AE, Mansur A, Graham CE, Bowman TA, Greenberg AS (2013) Perilipin-2-null mice are protected against diet-induced obesity, adipose inflammation, and fatty liver disease. *J Lipid Res* 54:1346–1359 1516 1517 1518
- Melo RCN, Weller PF (2016) Lipid droplets in leukocytes: organelles linked to inflammatory responses. *Exp Cell Res* 340:193–197 1519 1520
- Menon D, Singh K, Pinto SM, Nandy A, Jaisinghani N, Kutum R, Dash D, Prasad TSK, Gandotra S (2019) Quantitative lipid droplet proteomics reveals mycobacterium tuberculosis induced alterations in macrophage response to infection. *ACS Infect Dis* 5:559–569 1521 1522 1523
- Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Maréchal-Drouard L, Marshall WF, Qu L-H, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen C-L, Cognat V, Croft MT, Dent R, Dutcher S, Fernández E, Ferris P, Fukuzawa H, González-Ballester D, González-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanov M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral J-P, Riaño-Pachón DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen C-J, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT et al (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* 318:245–250 1524 1525 1526 1527 1528 1529 1530 1531 1532 1533 1534 1535 1536 1537 1538
- Meyer H, Bug M, Bremer S (2012) Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat Cell Biol* 14:117 1539 1540
- Missaglia S, Valadares ER, Moro L, Fagundes EDT, Quintão Roque R, Giardina B, Tavian D (2014) Early onset of Chanarin-Dorfman syndrome with severe liver involvement in a patient with a complex rearrangement of ABHD5 promoter. *BMC Med Genet* 15:32–32 1541 1542 1543
- Miura S, Gan J-W, Brzostowski J, Parisi MJ, Schultz CJ, Londos C, Oliver B, Kimmel AR (2002) Functional conservation for lipid storage droplet association among perilipin, ADRP, and TIP47 (PAT)-related proteins in mammals, *Drosophila*, and *Dictyostelium*. *J Biol Chem* 277:32253–32257 1544 1545 1546 1547
- Moellering ER, Benning C (2010) RNA interference silencing of a major lipid droplet protein affects lipid droplet size in *Chlamydomonas reinhardtii*. *Eukaryot Cell* 9:97–106 1548 1549

- 1550 Moore H-PH, Silver RB, Mottillo EP, Bernlohr DA, Granneman JG (2005) Perilipin targets a novel
 1551 pool of lipid droplets for lipolytic attack by hormone-sensitive lipase. *J Biol Chem*
 1552 280:43109–43120
- 1553 Morabito C, Bournaud C, Maës C, Schuler M, Aiese Cigliano R, Dellerio Y, Maréchal E, Amato A,
 1554 Rébeillé F (2019) The lipid metabolism in thraustochytrids. *Prog Lipid Res* 76:101007
- 1555 Możejko-Ciesielska J, Kiewisz R (2016) Bacterial polyhydroxyalkanoates: still fabulous?
 1556 *Microbiol Res* 192:271–282
- 1557 Müller AO, Blersch KF, Gippert AL, Ischebeck T (2016) Tobacco pollen tubes – a fast and easy
 1558 tool for studying lipid droplet association of plant proteins. *Plant J* 89:1055–1064
- 1559 Murphy DJ (2012) The dynamic roles of intracellular lipid droplets: from archaea to mammals.
 1560 *Protoplasma* 249:541–585
- 1561 Na H, Zhang P, Chen Y, Zhu X, Liu Y, Liu Y, Xie K, Xu N, Yang F, Yu Y, Cichello S, Mak HY,
 1562 Wang MC, Zhang H, Liu P (2015) Identification of lipid droplet structure-like/resident proteins
 1563 in *Caenorhabditis elegans*. *Biochim Biophys Acta* 1853:2481–2491
- 1564 Najt CP, Lwande JS, McIntosh AL, Senthivayagam S, Gupta S, Kuhn LA, Atshaves BP (2014)
 1565 Structural and functional assessment of perilipin 2 lipid binding domain(s). *Biochemistry*
 1566 53:7051–7066
- 1567 Nchoutmboube JA, Viktorova EG, Scott AJ, Ford LA, Pei Z, Watkins PA, Ernst RK, Belov GA
 1568 (2013) Increased long chain acyl-CoA synthetase activity and fatty acid import is linked to
 1569 membrane synthesis for development of picornavirus replication organelles. *PLoS Pathog* 9:
 1570 e1003401
- 1571 Nguyen HM, Baudet M, Cuiné S, Adriano J-M, Barthe D, Billon E, Bruley C, Beisson F, Peltier G,
 1572 Ferro M, Li-Beisson Y (2011) Proteomic profiling of oil bodies isolated from the unicellular
 1573 green microalga *Chlamydomonas reinhardtii*: with focus on proteins involved in lipid metab-
 1574 olism. *Proteomics* 11:4266–4273
- 1575 Nishino N, Tamori Y, Tateya S, Kawaguchi T, Shibakusa T, Mizunoya W, Inoue K, Kitazawa R,
 1576 Kitazawa S, Matsuki Y, Hiramatsu R, Masubuchi S, Omachi A, Kimura K, Saito M, Amo T,
 1577 Ohta S, Yamaguchi T, Osumi T, Cheng J, Fujimoto T, Nakao H, Nakao K, Aiba A, Okamura H,
 1578 Fushiki T, Kasuga M (2008) FSP27 contributes to efficient energy storage in murine white
 1579 adipocytes by promoting the formation of unilocular lipid droplets. *J Clin Invest* 118:2808–2821
- 1580 Nojima D, Yoshino T, Maeda Y, Tanaka M, Nemoto M, Tanaka T (2013) Proteomics analysis of oil
 1581 body-associated proteins in the oleaginous diatom. *J Proteome Res* 12:5293–5301
- 1582 Noothalapati Venkata HN, Shigeto S (2012) Stable isotope-labeled Raman imaging reveals
 1583 dynamic proteome localization to lipid droplets in single fission yeast cells. *Chem Biol*
 1584 19:1373–1380
- 1585 Okuda-Shimizu Y, Hendershot LM (2007) Characterization of an ERAD pathway for
 1586 non-glycosylated BiP substrates which requires Herp. *Mol Cell* 28:544–554
- 1587 Oliva J, French SW, Li J, Bardag-Gorce F (2012) Proteasome inhibitor treatment reduced fatty acid,
 1588 triacylglycerol and cholesterol synthesis. *Exp Mol Pathol* 93:26–34
- 1589 Olzmann JA, Kopito RR, Christianson JC (2013a) The mammalian endoplasmic reticulum-
 1590 associated degradation system. *Cold Spring Harb Perspect Biol*:5, a013185. [https://doi.org/10.](https://doi.org/10.1101/cshperspect.a013185)
 1591 [1101/cshperspect.a013185](https://doi.org/10.1101/cshperspect.a013185)
- 1592 Olzmann JA, Richter CM, Kopito RR (2013b) Spatial regulation of UBXD8 and p97/VCP controls
 1593 ATGL-mediated lipid droplet turnover. *Proc Natl Acad Sci USA* 110:1345–1350
- 1594 Onal G, Kutlu O, Gozuacik D, Dokmeci Emre S (2017) Lipid droplets in health and disease. *Lipids*
 1595 Health Dis 16:128
- 1596 Paar M, Jüngst C, Steiner NA, Magnes C, Sinner F, Kolb D, Lass A, Zimmermann R, Zumbusch A,
 1597 Kohlwein SD, Wolinski H (2012) Remodeling of lipid droplets during lipolysis and growth in
 1598 adipocytes. *J Biol Chem* 287:11164–11173
- 1599 Park S, Gidda SK, James CN, Horn PJ, Khuu N, Seay DC, Keereetaweep J, Chapman KD, Mullen
 1600 RT, Dyer JM (2013) The α/β hydrolase CGI-58 and peroxisomal transport protein PXA1
 1601 coregulate lipid homeostasis and signaling in Arabidopsis. *Plant Cell* 25:1726–1739

- Park S, Keereetaweep J, James CN, Gidda SK, Chapman KD, Mullen RT, Dyer JM (2014) CGI-58, a key regulator of lipid homeostasis and signaling in plants, also regulates polyamine metabolism. *Plant Signal Behav* 9:e27723
- Pasaribu B, Lin IP, Chen C-S, Lu C-Y, Jiang P-L (2014a) Nutrient limitation in *Auxenochlorella protothecoides* induces qualitative changes of fatty acid and expression of caleosin as a membrane protein associated with oil bodies. *Biotechnol Lett* 36:175–180
- Pasaribu B, Lin IP, Tzen JTC, Jauh G-Y, Fan T-Y, Ju Y-M, Cheng J-O, Chen C-S, Jiang P-L (2014b) SLDP: a novel protein related to caleosin is associated with the endosymbiotic symbiodinium lipid droplets from *Euphyllia glabrescens*. *Mar Biotechnol* 16:560–571
- Peled E, Leu S, Zarka A, Weiss M, Pick U, Khozin-Goldberg I, Boussiba S (2011) Isolation of a novel oil globule protein from the green alga *Haematococcus pluvialis* (Chlorophyceae). *Lipids* 46:851–861
- Peng S-E, Chen W-NU, Chen H-K, Lu C-Y, Mayfield AB, Fang L-S, Chen C-S (2011) Lipid bodies in coral–dinoflagellate endosymbiosis: proteomic and ultrastructural studies. *Proteomics* 11:3540–3555
- Pericleous M, Kelly C, Wang T, Livingstone C, Ala A (2017) Wolman’s disease and cholesteryl ester storage disorder: the phenotypic spectrum of lysosomal acid lipase deficiency. *Lancet Gastroenterol Hepatol* 2:670–679
- Petroutsos D, Amiar S, Abida H, Dolch L-J, Bastien O, Rébeillé F, Jouhet J, Falconet D, Block MA, Mcfadden GI, Bowler C, Botté C, Maréchal E (2014) Evolution of galactoglycerolipid biosynthetic pathways – from cyanobacteria to primary plastids and from primary to secondary plastids. *Prog Lipid Res* 54:68–85
- Pol A, Gross SP, Parton RG (2014) Biogenesis of the multifunctional lipid droplet: lipids, proteins, and sites. *J Cell Biol* 204:635–646
- Popluechai S, Froissard M, Jolivet P, Breviario D, Gatehouse AMR, O’donnell AG, Chardot T, Kohli A (2011) *Jatropha curcas* oil body proteome and oleosins: L-form JcOle3 as a potential phylogenetic marker. *Plant Physiol Biochem* 49:352–356
- Pötter M, Steinbüchel A (2006) Biogenesis and structure of polyhydroxyalkanoate granules. In: Shively JM (ed) *Inclusions in prokaryotes*. Springer, Berlin, pp 109–136
- Poulsen LLC, Siersbæk M, Mandrup S (2012) PPARs: fatty acid sensors controlling metabolism. *Semin Cell Dev Biol* 23:631–639
- Qu R, Wang SM, Lin YH, Vance VB, Huang AH (1986) Characteristics and biosynthesis of membrane proteins of lipid bodies in the scutella of maize (*Zea mays* L.). *Biochem J* 235:57–65
- Quettier A-L, Eastmond PJ (2009) Storage oil hydrolysis during early seedling growth. *Plant Physiol Biochem* 47:485–490
- Rapoport TA (2007) Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. *Nature* 450:663
- Reinecke F, Steinbüchel A (2009) *Ralstonia eutropha* strain H16 as model organism for PHA metabolism and for biotechnological production of technically interesting biopolymers. *J Mol Microbiol Biotechnol* 16:91–108
- Roingeard P, Melo RCN (2017) Lipid droplet hijacking by intracellular pathogens. *Cell Microbiol* 19:e12688
- Ruggiano A, Mora G, Buxó L, Carvalho P (2016) Spatial control of lipid droplet proteins by the ERAD ubiquitin ligase Doa10. *EMBO J* 35:1644–1655
- Rumin J, Bonnefond H, Saint-Jean B, Rouxel C, Sciandra A, Bernard O, Cadoret J-P, Bougaran G (2015) The use of fluorescent Nile red and BODIPY for lipid measurement in microalgae. *Biotechnol Biofuels* 8:42
- Rydel TJ, Williams JM, Krieger E, Moshiri F, Stallings WC, Brown SM, Pershing JC, Purcell JP, Alibhai MF (2003) The crystal structure, mutagenesis, and activity studies reveal that patatin is a lipid acyl hydrolase with a Ser-Asp catalytic dyad. *Biochemistry* 42:6696–6708
- Sahu-Osen A, Montero-Moran G, Schittmayer M, Fritz K, Dinh A, Chang Y-F, McMahon D, Boeszoermenyi A, Cornaciu I, Russell D, Oberer M, Carman GM, Birner-Gruenberger R,

- 1654 Brasaemle DL (2015) CGI-58/ABHD5 is phosphorylated on Ser239 by protein kinase a: control
1655 of subcellular localization. *J Lipid Res* 56:109–121
- 1656 Saka HA, Thompson JW, Chen Y-S, Dubois LG, Haas JT, Moseley A, Valdivia RH (2015)
1657 Chlamydia trachomatis infection leads to defined alterations to the lipid droplet proteome in
1658 epithelial cells. *PLoS One* 10:e0124630
- 1659 Salo VT, Belevich I, Li S, Karhinen L, Vihinen H, Vigouroux C, Magré J, Thiele C, Hölttä-Vuori M,
1660 Jokitalo E, Ikonen E (2016) Seipin regulates ER–lipid droplet contacts and cargo delivery.
1661 *EMBO J* 35:2699–2716
- 1662 Sando T, Hayashi T, Takeda T, Akiyama Y, Nakazawa Y, Fukusaki E, Kobayashi A (2009)
1663 Histochemical study of detailed laticifer structure and rubber biosynthesis-related protein
1664 localization in *Hevea brasiliensis* using spectral confocal laser scanning microscopy. *Planta*
1665 230:215–225
- 1666 Sardet C (2013) Plancton - Aux origines du vivant
- 1667 Schweiger M, Paar M, Eder C, Brandis J, Moser E, Gorkiewicz G, Grond S, Radner FPW, Cerk I,
1668 Cornaciu I, Oberer M, Kersten S, Zechner R, Zimmermann R, Lass A (2012) G0/G1 switch
1669 gene-2 regulates human adipocyte lipolysis by affecting activity and localization of adipose
1670 triglyceride lipase. *J Lipid Res* 53:2307–2317
- 1671 Sharma B, Joshi D, Yadav PK, Gupta AK, Bhatt TK (2016) Role of ubiquitin-mediated degradation
1672 system in plant biology. *Front Plant Sci* 7:806
- 1673 Shen P, Zhao Q, Yao C, Wu S, Meng Y, Zhang L, Xue S (2016) Differential proteome analysis and
1674 identification of lipid droplet associated proteins in the marine microalgae *Isochrysis*
1675 *zhangjiangensis* (Haptophyta). In: The 4th Asia-Oceania algae innovation summit. Wuhan
- 1676 Shi Q (2019) Expression profiling of genes coding for abundant proteins in the alkenone body of
1677 marine haptophyte alga *Tisochrysis lutea*. *BMC Microbiol* 19:56–56
- 1678 Shi X, Li J, Zou X, Greggain J, Rødkær SV, Færgeman NJ, Liang B, Watts JL (2013) Regulation of
1679 lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. *J Lipid Res*
1680 54:2504–2514
- 1681 Shi Q, Araie H, Bakku RK, Fukao Y, Rakwal R, Suzuki I, Shiraiwa Y (2015) Proteomic analysis of
1682 lipid body from the alkenone-producing marine haptophyte alga *Tisochrysis lutea*. *Proteomics*
1683 15:4145–4158
- 1684 Shimada TL, Hara-Nishimura I (2015) Leaf oil bodies are subcellular factories producing antifungal
1685 oxylipins. *Curr Opin Plant Biol* 25:145–150
- 1686 Shimada TL, Takano Y, Shimada T, Fujiwara M, Fukao Y, Mori M, Okazaki Y, Saito K, Sasaki R,
1687 Aoki K, Hara-Nishimura I (2014) Leaf oil body functions as a subcellular factory for the
1688 production of a Phytoalexin in Arabidopsis. *Plant Physiol* 164:105–118
- 1689 Shimada TL, Takano Y, Hara-Nishimura I (2015) Oil body-mediated defense against fungi: from
1690 tissues to ecology. *Plant Signal Behav* 10:e989036
- 1691 Siegler H, Valerius O, Ischebeck T, Popko J, Tourasse NJ, Vallon O, Khozin-Goldberg I, Braus
1692 GH, Feussner I (2017) Analysis of the lipid body proteome of the oleaginous alga *Lobosphaera*
1693 *incisa*. *BMC Plant Biol* 17:98
- 1694 Song P, Li L, Liu J (2013) Proteomic analysis in nitrogen-deprived *Isochrysis galbana* during lipid
1695 accumulation. *PLoS One* 8:e82188–e82188
- 1696 Soni KG, Mardones GA, Sougrat R, Smirnova E, Jackson CL, Bonifacino JS (2009) Coatomer-
1697 dependent protein delivery to lipid droplets. *J Cell Sci* 122:1834
- 1698 Soulages JL, Firdaus SJ, Hartson S, Chen X, Howard AD, Arrese EL (2012) Developmental
1699 changes in the protein composition of *Manduca sexta* lipid droplets. *Insect Biochem Mol Biol*
1700 42:305–320
- 1701 Souza SC, De Vargas LM, Yamamoto MT, Lien P, Franciosa MD, Moss LG, Greenberg AS (1998)
1702 Overexpression of Perilipin A and B blocks the ability of tumor necrosis factor α to increase
1703 lipolysis in 3T3-L1 adipocytes. *J Biol Chem* 273:24665–24669
- 1704 Stevenson J, Huang EY, Olzmann JA (2016) Endoplasmic reticulum-associated degradation and
1705 lipid homeostasis. *Annu Rev Nutr* 36:511–542

- Stone SJ, Levin MC, Zhou P, Han J, Walther TC, Farese RV (2009) The endoplasmic reticulum enzyme DGAT2 is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria. *J Biol Chem* 284:5352–5361
- Subramanian V, Rothenberg A, Gomez C, Cohen AW, Garcia A, Bhattacharyya S, Shapiro L, Dolios G, Wang R, Lisanti MP, Brasaemle DL (2004) Perilipin 1 mediates the reversible binding of CGI-58 to lipid droplets in 3T3-L1 adipocytes. *J Biol Chem* 279:42062–42071
- Sun Z, Gong J, Wu H, Xu W, Wu L, Xu D, Gao J, Wu J-W, Yang H, Yang M, Li P (2013) Perilipin1 promotes unilocular lipid droplet formation through the activation of Fsp27 in adipocytes. *Nat Commun* 4:1594
- Suzuki M, Otsuka T, Ohsaki Y, Cheng J, Taniguchi T, Hashimoto H, Taniguchi H, Fujimoto T (2012) Derlin-1 and UBXD8 are engaged in dislocation and degradation of lipidated ApoB-100 at lipid droplets. *Mol Biol Cell* 23:800–810
- Sznajder A, Jendrossek D (2014) To be or not to be a poly(3-hydroxybutyrate) (PHB) depolymerase: PhaZd1 (PhaZ6) and PhaZd2 (PhaZ7) of *Ralstonia eutropha*, highly active PHB depolymerases with no detectable role in mobilization of accumulated PHB. *Appl Environ Microbiol* 80:4936–4946
- Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng CX, Li C, Kimmel AR, Londos C (2001) Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc Natl Acad Sci* 98:6494
- Tauchi-Sato K, Ozeki S, Houjou T, Taguchi R, Fujimoto T (2002) The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition. *J Biol Chem* 277:44507–44512
- Taurino M, Costantini S, De Domenico S, Stefanelli F, Ruano G, Delgadillo MO, Sanchez-Serrano JJ, Sanmartín M, Santino A, Rojo E (2017) SEIPIN proteins mediate lipid droplet biogenesis to promote pollen transmission and reduce seed dormancy. *Plant Physiol* 176:1531–1546
- Theodoulou FL, Job K, Slocombe SP, Footitt S, Holdsworth M, Baker A, Larson TR, Graham IA (2005) Jasmonic acid levels are reduced in COMATOSE ATP-binding cassette transporter mutants. Implications for transport of jasmonate precursors into peroxisomes. *Plant Physiol* 137:835–840
- Thiam AR, Forêt L (2016) The physics of lipid droplet nucleation, growth and budding. *Biochim Biophys Acta* 1861:715–722
- Thiam AR, Antonny B, Wang J, Delacotte J, Wilfling F, Walther TC, Beck R, Rothman JE, Pincet F (2013a) COPI buds 60-nm lipid droplets from reconstituted water–phospholipid–triacylglyceride interfaces, suggesting a tension clamp function. *Proc Natl Acad Sci USA* 110:13244–13249
- Thiam AR, Farese RV Jr, Walther TC (2013b) The biophysics and cell biology of lipid droplets. *Nat Rev Mol Cell Biol* 14:775
- Thul PJ, Tschapalda K, Kolkhof P, Thiam AR, Oberer M, Beller M (2017) Targeting of the *Drosophila* protein CG2254/Ldsdh1 to a subset of lipid droplets. *J Cell Sci* 130:3141
- Tnani H, López I, Jouenne T, Vicient CM (2011) Protein composition analysis of oil bodies from maize embryos during germination. *J Plant Physiol* 168:510–513
- Trentacoste EM, Shrestha RP, Smith SR, Glé C, Hartmann AC, Hildebrand M, Gerwick WH (2013) Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth. *Proc Natl Acad Sci USA* 110:19748–19753
- Uchino K, Saito T, Jendrossek D (2008) Poly(3-hydroxybutyrate) (PHB) depolymerase PhaZa1 is involved in mobilization of accumulated PHB in *Ralstonia eutropha* H16. *Appl Environ Microbiol* 74:1058–1063
- Van Wijk KJ, Kessler F (2017) Plastoglobuli: plastid microcompartments with integrated functions in metabolism, plastid developmental transitions, and environmental adaptation. *Annu Rev Plant Biol* 68:253–289
- Vance VB, Huang AH (1987) The major protein from lipid bodies of maize. Characterization and structure based on cDNA cloning. *J Biol Chem* 262:11275–11279

- 1758 Varela GM, Antwi DA, Dhir R, Yin X, Singhal NS, Graham MJ, Crooke RM, Ahima RS (2008)
 1759 Inhibition of ADRP prevents diet-induced insulin resistance. *Am J Physiol Gastrointest Liver*
 1760 *Physiol* 295:G621–G628
- 1761 Vermachova M, Purkrtova Z, Santrucek J, Jolivet P, Chardot T, Kodicek M (2011) New protein
 1762 isoforms identified within *Arabidopsis thaliana* seed oil bodies combining chymotrypsin/
 1763 trypsin digestion and peptide fragmentation analysis. *Proteomics* 11:3430–3434
- 1764 Vieler A, Wu G, Tsai C-H, Bullard B, Cornish AJ, Harvey C, Reca I-B, Thornburg C,
 1765 Achawanantakun R, Buehl CJ, Campbell MS, Cavalier D, Childs KL, Clark TJ,
 1766 Deshpande R, Erickson E, Armenia Ferguson A, Handee W, Kong Q, Li X, Liu B,
 1767 Lundback S, Peng C, Roston RL, Sanjaya, Simpson JP, Terbush A, Warakanont J, Zäuner S,
 1768 Farre EM, Hegg EL, Jiang N, Kuo M-H, Lu Y, Niyogi KK, Ohlrogge J, Osteryoung KW,
 1769 Shachar-Hill Y, Sears BB, Sun Y, Takahashi H, Yandell M, Shiu S-H, Benning C (2012)
 1770 Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous
 1771 alga *Nannochloropsis oceanica* CCMP1779. *PLoS Genet* 8:e1003064
- 1772 Viswanadha S, Londos C (2008) Determination of lipolysis in isolated primary adipocytes. In:
 1773 Yang K (ed) *Adipose tissue protocols*. Humana Press, Totowa, pp 299–306
- 1774 Vrablik TL, Petyuk VA, Larson EM, Smith RD, Watts JL (2015) Lipidomic and proteomic analysis
 1775 of *C. elegans* lipid droplets and identification of ACS-4 as a lipid droplet-associated protein.
 1776 *Biochim Biophys Acta* 1851:1337–1345
- 1777 Wältermann M, Steinbüchel A (2005) Neutral lipid bodies in prokaryotes: recent insights into
 1778 structure, formation, and relationship to eukaryotic lipid depots. *J Bacteriol* 187:3607–3619
- 1779 Walther TC, Chung J, Farese RV (2017) Lipid droplet biogenesis. *Annu Rev Cell Dev Biol*
 1780 33:491–510
- 1781 Wang H, Becuwe M, Housden BE, Chitraju C, Porras AJ, Graham MM, Liu XN, Thiam AR,
 1782 Savage DB, Agarwal AK, Garg A, Olarte M-J, Lin Q, Fröhlich F, Hannibal-Bach HK,
 1783 Upadhyayula S, Perrimon N, Kirchhausen T, Ejsing CS, Walther TC, Farese RV (2016) Seipin
 1784 is required for converting nascent to mature lipid droplets. *elife* 5:e16582
- 1785 Watanabe T, Sakiyama R, Iimi Y, Sekine S, Abe E, Nomura KH, Nomura K, Ishibashi Y, Okino N,
 1786 Hayashi M, Ito M (2017) Regulation of TG accumulation and lipid droplet morphology by the
 1787 novel TLDPI in *Aurantiochytrium limacinum* F26-b. *J Lipid Res* 58:2334–2347
- 1788 Wilfling F, Wang H, Haas JT, Krahmer N, Gould TJ, Uchida A, Cheng J-X, Graham M,
 1789 Christiano R, Fröhlich F, Liu X, Buhman KK, Coleman RA, Bewersdorf J, Farese RV, Walther
 1790 TC (2013) Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocating from
 1791 the ER to lipid droplets. *Dev Cell* 24:384–399
- 1792 Wilfling F, Haas JT, Walther TC, Farese RV (2014) Lipid droplet biogenesis. *Curr Opin Cell Biol*
 1793 29:39–45
- 1794 Wolman M, Sterk VV, Gatt S, Frenkel M (1961) Primary familial xanthomatosis with involvement
 1795 and calcification of the adrenals. Report of two more cases in siblings of a previously described
 1796 infant. *Pediatrics* 25:742–757
- 1797 Xie M, Roy R (2015) The causative gene in Chanarian Dorfman syndrome regulates lipid droplet
 1798 homeostasis in *C. elegans*. *PLoS Genet* 11:e1005284–e1005284
- 1799 Yang H-J, Hsu C-L, Yang J-Y, Yang WY (2012) Monodansylpentane as a blue-fluorescent lipid-
 1800 droplet marker for multi-color live-cell imaging. *PLoS One* 7:e32693
- 1801 Yermanos DM (1975) Composition of jojoba seed during development. *J Am Oil Chem Soc*
 1802 52:115–117
- 1803 Yoneda K, Yoshida M, Suzuki I, Watanabe MM (2016) Identification of a major lipid droplet
 1804 protein in a marine diatom *Phaeodactylum tricorutum*. *Plant Cell Physiol* 57:397–406
- 1805 Yoneda K, Yoshida M, Suzuki I, Watanabe MM (2018) Homologous expression of lipid droplet
 1806 protein-enhanced neutral lipid accumulation in the marine diatom *Phaeodactylum tricorutum*.
 1807 *J Appl Phycol* 30:2793–2802
- 1808 Youssef A, Laizet YH, Block MA, Maréchal E, Alcaraz J-P, Larson TR, Pontier D, Gaffé J, Kuntz
 1809 M (2010) Plant lipid-associated fibrillin proteins condition jasmonate production under photo-
 1810 synthetic stress. *Plant J* 61:436–445

- Yu J, Zhang S, Cui L, Wang W, Na H, Zhu X, Li L, Xu G, Yang F, Christian M, Liu P (2015) Lipid droplet remodeling and interaction with mitochondria in mouse brown adipose tissue during cold treatment. *Biochim Biophys Acta* 1853:918–928
- Yu Y, Li T, Wu N, Jiang L, Ji X, Huang H (2017) The role of lipid droplets in *Mortierella alpina* aging revealed by integrative subcellular and whole-cell proteome analysis. *Sci Rep* 7:43896
- Zhang C, Liu P (2017) The lipid droplet: a conserved cellular organelle. *Protein Cell* 8:796–800
- Zhang P, Na H, Liu Z, Zhang S, Xue P, Chen Y, Pu J, Peng G, Huang X, Yang F, Xie Z, Xu T, Xu P, Ou G, Zhang SO, Liu P (2012) Proteomic study and marker protein identification of *Caenorhabditis elegans* lipid droplets. *Mol Cell Proteomics* 11:317–328
- Zhang J, Zhang Z, Chukkapalli V, Nchoutmboube JA, Li J, Randall G, Belov GA, Wang X (2016) Positive-strand RNA viruses stimulate host phosphatidylcholine synthesis at viral replication sites. *Proc Natl Acad Sci USA* 113:E1064–E1073
- Zhang C, Yang L, Ding Y, Wang Y, Lan L, Ma Q, Chi X, Wei P, Zhao Y, Steinbüchel A, Zhang H, Liu P (2017) Bacterial lipid droplets bind to DNA via an intermediary protein that enhances survival under stress. *Nat Commun* 8:15979
- Zhu Z, Ding Y, Gong Z, Yang L, Zhang S, Zhang C, Lin X, Shen H, Zou H, Xie Z, Yang F, Zhao X, Liu P, Zhao ZK (2015) Dynamics of the lipid droplet proteome of the oleaginous yeast *Rhodospiridium toruloides*. *Eukaryot Cell* 14:252–264
- Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 306:1383–1386

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