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Chapter 11

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

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Josselin Lupette and Eric Maréchal

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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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[AU1](#)

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

32 **Keywords** Lipid droplets · Evolution · Architecture · Biogenesis · Catabolism

11.1 Introduction

[AU2](#)

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

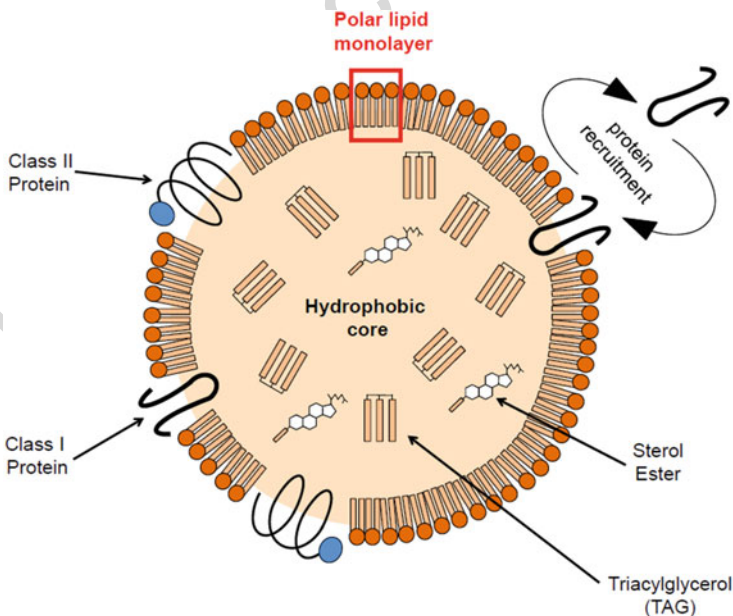


Fig. 11.1 General architecture of a triacylglycerol-containing lipid droplet

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.

AU3

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

t1.1 **Table 11.1** Human diseases related to LD formation

AU4

t1.2	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), Samueloy 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)

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

96 TAG-rich LDs produced by oleaginous organisms, mainly plants and algae, but
 97 sometimes also fungi or animals, are also key to numerous biotechnological appli-
 98 cations. Molecules of TAG are composed of a glycerol-3-phosphate backbone on
 99 which three fatty acids are esterified (Lupette and Maréchal 2018). Fatty acids (FAs)
 100 are carboxylic acids. Their carbon chain length and number of unsaturations
 101 (or double bonds, C=C) allow assessing whether they can be used for different
 102 applications. Oleaginous crops are an essential resource for human nutrition.
 103 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 116

11.2.1 Imaging Lipid Droplets 117

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 tricoratum* (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 130

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

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

150 **11.2.3 Biophysical Properties of Lipid Droplets**

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

172 **11.3 Lipid Droplets in the Tree of Life**

173 **11.3.1 Lipid Droplets in Prokaryotic Cells**

174 The vast majority of bacterial species have the capacity to accumulate lipid com-
175 pounds 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 (Mozejko-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 (Mozejko-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 (Roingard 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,

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

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

305 11.3.3.1 *Opisthokonta: Non-photosynthetic Eukaryotes*

306 Metazoa

307 *Chordata*

308 LDs of mammals are composed of a hydrophobic core consisting of TAGs and
309 cholesteryl esters, generally considered as a form of storage. The monolayer of polar
310 lipids is mainly composed of PC, PE, and PI (Bartz et al. 2007). In mammals, LD
311 surface proteins are grouped into a family formerly called PAT (for Perilipin—
312 Adipophilin—Tail-interacting protein of 47 kDa) (Bickel et al. 2009) but whose
313 nomenclature has evolved since 2010 under the name of Perilipin (PLIN) (Kimmel
314 et al. 2010). PLIN1 has four splice variants PLIN1a, PLIN1b, PLIN1c, and PLIN1d
315 (Kimmel et al. 2010). PLINs contain a hydrophobic PAT domain of 100 amino acids
316 defining an N-terminal region. The study of a mutant PLIN2-N1 (deficient for the
317 PAT domain) reveals that this domain is not involved in lipid binding, but in the
318 stabilization of lipid droplets, and lipid accumulation and degradation of PLIN2 by
319 the proteasome (Najt et al. 2014). The PAT domain is followed by an 11-mer helical
320 pattern of variable size, which might interact with phospholipids (Bussell and Eliezer
321 2003). Some PLIN proteins can be post-translationally modified by phosphoryla-
322 tions, via PKA, during lipolysis (PLIN3 and PLIN4 do not have a phosphorylation
323 site (Kimmel and Sztalryd 2016)). Recently, the sixth clade of Perilipin, called
324 PLIN6, has been discovered, specific to teleosts (Granneman et al. 2017). Interest-
325 ingly, PLIN6 is not expressed in tissues associated with lipid metabolism but in the
326 xanthophores of teleost skin. Biochemical analyses have shown that PLIN6 is
327 associated with the surface of droplets enriched in carotenoids and regulates the
328 pigment synthesis pathways (Granneman et al. 2017).

329 The expression of PLINs (with the exception of PLIN3) is regulated by a family
330 of transcription factors called PPAR (Peroxisome Proliferator Activated Receptor).
331 These transcription factors are activated by the binding of lipid ligands (Poulsen
332 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	Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References		
Prokaryota	Proteobacteria	<i>Cupriavidus necator</i>	PhaC/PhaP/ PhaR/PhaZ	PHB granules	Bresan et al. (2016), Peoples et al. (1989), Schubert et al. (1988), Slater et al. (1988), Germgross et al. (1993), Dennis et al. (1998), Tariq et al. (2015), Han et al. (2007)		
			β-Proteobacteria				
	Actinobacteria	γ-Proteobacteria	<i>Marinobacter</i>	TAGs		Kalscheuer et al. (2007)	
			<i>Alcantivorax</i>				
		Mycobacteriaceae	<i>Mycobacterium tuberculosis</i>	PLIN2, PLIN3, ARL8B	TAGs	Pathogenic bacteria	Menon et al. (2019), Kim et al. (2010), Caire-Brändli et al. (2014), Melo and Weller et al. (2005), Luo et al. (2010)
			<i>Mycobacterium bovis</i> /BCG	Tgs1, Tgs2, BCG1169c, BCG1489c, BCG1721			
			<i>Mycobacterium avium</i>				
	Nocardiaceae	<i>Rhodococcus jostii</i>	MLDS/Psp A	TAGs		Ding et al. (2012a, b)	
		<i>Rhodococcus opacus</i>	LPD06283			Kalscheuer et al. (2001), Chen et al. (2014)	
		<i>Rhodococcus ruber</i>	Ribosomal protein L7			Kalscheuer et al. (2001)	
	Chlamydiae	<i>Chlamydia trachomatis</i>	PLIN2, PLIN2, ACSL-3, ACSL-4, CGI-58	TAGs	Pathogenic bacteria	Saka et al. (2015)	

AU7 t2.1

t2.2

t2.3

t2.4

t2.7

AU8 t2.8

t2.6

AU9 t2.9

t2.10

t2.11

t2.12

11 The Puzzling Conservation and Diversification of Lipid Droplets from Bacteria . . .

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>Cricentulus griseus</i>	PLINs, CGI-58, ATGL, HSL, CNX	TAGs/SE		Batz et al. (2007), Bickel et al. (2009), Kimmel et al. (2010), Greenberg et al. (1991), Orficky et al. (2008), Najt et al. (2014), Bussell and Eliezer (2003), Hickenbottom et al. (2004), Kimmel and Szialyrd (2016), Granneman et al. (2017), Mandard et al. (2004), Poulsen et al. (2012), Targett-Adams et al. (2005), Dalen et al. (2007), Edwardsson 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)

t2.17 Phylogeny		Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References
		<i>Mus musculus</i>				Wu et al. (2000), Brasaemle 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)
t2.15		<i>Rattus norvegicus</i>				Turro et al. (2006), Larsson et al. (2012), Eichmann et al. (2015), Khor et al. (2014)
t2.16		<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)

	Phylogeny	Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References
t2.31		<i>Schizosaccharomyces pombe</i>		TAG/SE		Venkata and Shigeto (2012)
t2.29		<i>Rhodospiridium toruloides</i>				Zhu et al. (2015)
t2.30		<i>Dyctostelium discoideum</i>	plnA	TAGs/FFA		Du et al. (2013)
t2.31	Amoebozoa					
t2.32	Excavata	<i>Trypanozoma cruzi</i>			LDs induction	D'Avila et al. (2011)
t2.33		<i>Leishmania amazonensis</i>				Lecoq 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-oleosin, pol-len—U oleosin, Caleosin, Stereoleosin	TAGs	OLDs (seeds, pollen, leaves)	Jolivet et al. (2004), Vermachova et al. (2011)
t2.36		<i>Brassica napus</i>				Katavic 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>Gevunia 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	TAGs			Tapetosome	Huang (2018)
t2.46	<i>Persea americana</i>	LDAP1, LDAP2, M-oleosin	TAGs			OLDs—mesocarp	Horn et al. (2013)
t2.47	<i>Allium cepa</i>	U-oleosin/caleosin	TAGs			OLDs—LDs cluster	Huang (2018)
t2.48	<i>Vanilla planifolia</i>	U-oleosin					
t2.49	<i>Aloe vera</i>	U-oleosin					
t2.50	<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)
t2.51	<i>Capsicum annuum</i>	PAP/Fibrillin	Yes			Plastoglobules—NOLDs	Van Wijk and Kessler (2017)
t2.52	<i>Chlamydomonas reinhardtii</i>	MLDP	TAGs				Moellering and Benning (2010), Nguyen et al. (2011), James et al. (2011)
t2.53	<i>Dunaliella bardawil</i>		TAGs				Davidi et al. (2012)
t2.55	<i>Dunaliella salina</i>						
t2.56	<i>Dunaliella parva</i>						
t2.57	<i>Scenedesmus quadricauda</i>		TAGs				Javee et al. (2016)
t2.58	<i>Haematococcus pluvialis</i>	HOGP	TAGs/DCGTS/PC/SQDG/DGDDG			Pigments in LDs	Peled et al. (2011)
	<i>Chlorella sp.</i>	Caleosin	TAGs/DAG				Lin et al. (2012)
	<i>Lobosphaera incisa</i>		TAGs				

(continued)

t2.60 **Table 11.2** (continued)

t2.61	Phylogeny		Species	LD Proteome/ LD protein characterized	LD lipidome	Other informations	References
t2.59	Chromista	Haptista	<i>Tisochrysis lutea</i>	LiMLDP, LiLBP62, LiLBP36	Alkenone	Alkenone body	Siegler et al. (2017), Bigogno et al. (2002)
t2.60		Heterokonta		Yes			Marlowe et al. (1984a), Marlowe et al. (1984b), Song et al. (2013), Shi et al. (2015), Shi et al. (2019)
t2.61	SAR super- group/ Harosa	Alvaria	<i>Nannochloropsis oceanica</i>	LDSP	TAGs		Vielser et al. (2012)
t2.62			<i>Fistulifera solaris</i>	DOAP1	TAGs		Nojima et al. (2013)
t2.63			<i>Phaeodactylum tricornutum</i>	SLDP	TAGs/DGTA/ SQDG/PC		Yoneda et al. (2016), Lupette et al. (2019)
t2.64				<i>Aurantiochytrium limacinum</i>	TLDP1	TAGs	
t2.65	Alveolata	Apicomplexa Dinophyta	<i>Toxoplasma gondii</i>	SLDP	TAGs/CE	LDs induction	Hu et al. (2017)
t2.66				<i>Symbiodinium</i> sp.			
t2.67	Rhizaria	Cercozoa	<i>Plasmodiophora brassicaceae</i>	Yes	TAGs		Bi et al. (2016)
t2.68	Retaria		<i>Ammonia tepida</i>			LDs detections	LeKieffre et al. (2017), Le Cadre et al. (2006)
t2.69				<i>Ammonia beccarii</i>			

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

419 nematode has LDs measuring 1–1.5 μm in diameter in its intestine and in the
420 hypodermis. Three proteomic studies of *C. elegans* LDs have been performed
421 (Zhang et al. 2012; Na et al. 2015; Vrablik et al. 2015). The first shotgun proteomic
422 analysis allowed the identification of 306 proteins of which 193 were known to be
423 associated with mammalian LDs (Zhang et al. 2012). This first study identified the
424 DHS-3 protein on the surface of the LD *via* a GFP fusion. A second proteome of the
425 LD of *C. elegans* allowed the identification of 154 proteins of which 113 are common
426 with the first proteome (Na et al. 2015). DHS-3 and MDT-28 are the two major
427 proteins in *C. elegans* LD. The deletion of the *dhs-3* gene causes a decrease in the
428 size of LDs as well as the amount of their TAG. The *mdt-28* mutant causes the
429 formation of LD aggregations (Na et al. 2015). A third proteome compared the LD
430 protein composition of a *C. elegans* wild type and high *daf-2* (e1370) fat mutant
431 (Vrablik et al. 2015). Using a GFP construct, the ACS-4 protein, an acyl-CoA
432 synthase, was localized at the surface of the *C. elegans* LD. It has long been thought
433 that PLINs were lost in *C. elegans*. However, three isoforms of mammalian PLINs
434 have been identified: PLIN-1a, PLIN-1b, and PLIN-1c. These isoforms have an
435 N-terminal PAT domain, an amphiphilic region with imperfect helices, and four
436 C-terminal helices (Chughtai et al. 2015). The *C. elegans* genome seems to also code
437 for several sequences of the LD protein actors ABHD4 (*CeLid-1*) and ABHD5/CGI-
438 58 (*CeAbhd5.2*) (Lee et al. 2014; Xie and Roy 2015).

439 Fungi

440 *Saccharomyces cerevisiae* is a widely used model for studying lipid biology because
441 the synthetic pathways in the ER are similar to those of plants and animals (Koch
442 et al. 2014). LDs measure about 400 nm in this organism. The hydrophobic core of
443 LDs of *S. cerevisiae* is composed of TAGs grouped in the center and surrounded by
444 the steryl ester molecules (Leber et al. 1994; Czabany et al. 2008). There is also a
445 minor proportion of squalene and sterols. The monolayer of phospholipids consists
446 of PC, PI, PE, PA, and PS (Tauchi-Sato et al. 2002; Grillitsch et al. 2011). The
447 proteome of the LD highlighted proteins that contribute to the synthesis of the
448 hydrophobic core, such as sterol- $\Delta 24$ -methyltransferase, squalene epoxidase, and
449 lanosterol synthetase (Leber et al. 1994; Athenstaedt et al. 1999) (Table 11.2). In
450 *Pichia pastoris*, the polar lipid monolayer is mainly composed of PC and PE but
451 there is also a lower proportion of PI, PS, PA, cardiolipin, lysophospholipids, and
452 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) (Ivashov et al. 2013)
453 (Table 11.2). Studies have also been conducted in other models such as *Yarrowia*
454 *lipolytica* (Athenstaedt et al. 2006), *Mortierella alpina* (Yu et al. 2017), *Cryptococ-*
455 *cus albidus* (Shi et al. 2013), *Trichosporon fermentans* (Shen et al. 2016),
456 *Schizosaccharomyces pombe* (Noothalapati Venkata and Shigeto 2012), or
457 *Rhodospiridium toruloides* (Zhu et al. 2015). Identified proteins comprise a majority
458 of orthologues of *S. cerevisiae* and one can deduce from this analysis the enzymatic
459 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

499 long been considered a unique event during evolution. The study of *Paulinella*
500 *chromatophora* (a photosynthetic amoeba) has shown that a second, more recent,
501 primary endosymbiosis event (60–100 million years ago) occurred between
502 cyanobacteria and an amoeba (Maréchal 2018). This endosymbiosis led to the
503 formation of an organelle also limited by two membranes, called the chromatophore.

504 In contrast to non-photosynthetic eukaryotes, in which FA biosynthesis occurs in
505 the cytosol, FAs are synthesized in the stroma of the chloroplast and then exported to
506 the cytosol. On the one hand, chloroplasts contain LD, called plastoglobules. On the
507 other hand, the plastid appears to play a role in the production of TAG and
508 biogenesis of cytosolic LDs in some of the lineages of photosynthetic eukaryotes.
509 Stronger cooperation of the ER and plastid in LD formation may therefore be an
510 important innovation in these primary endosymbionts. This may also be related to
511 the loss of perilipins and the emergence of specific LD-associated proteins. Our
512 understanding of LD evolution in primary endosymbionts is mainly based on
513 analyses performed in Chlorophyta and land plants (Embryophyta, mostly in
514 Angiosperms).

515 Chlorophyta

516 *Chlamydomonas reinhardtii* is a green alga that accumulates oils in the form of LDs
517 following environmental stresses such as a nitrogen deficiency or an increase in
518 salinity. A proteomic study of LD performed in *C. reinhardtii* highlighted a Major
519 Lipid Droplet Protein (MLDP) of 27 kDa (Moellering and Benning 2010; James
520 et al. 2011; Nguyen et al. 2011). The phenotype of a *mldp* mutant suggests that
521 MLDP is involved in the regulation of LD size (Moellering and Benning 2010). A
522 33 kDa homolog of MLDP was also found in *Haematococcus pluvialis* (Peled et al.
523 2011) as well as in three *Dunaliella* species (Davidi et al. 2012). MLDP was also
524 detected in *Scenedesmus quadricauda* during salt stress or nitrogen deficiency
525 (Javee et al. 2016). MLDP orthologues are also present in several species of
526 Chlorophyta: *Volyox carteri*, *Haematococcus pluvialis*, *Dunaliella salina*,
527 *Coccomyxa* sp., *Chlorella variabilis*, *Polytomella parva*, *Prototheca wickerhamii*,
528 and *Micromonas pusilla* CCMP1545 (Goold et al. 2015). A 28 kDa caleosin protein
529 was shown to be the major protein in the LD of *Chlorella* sp. (Lin et al. 2012). The
530 size of LDs of *Chlorella* can reach 3 µm (Lin et al. 2012). The caleosin localization
531 was specifically determined on the surface of the LD by immunostaining with gold
532 beads (Pasaribu et al. 2014a). In these studies, caleosins first characterized in plants
533 (see below) seem to be conserved LD-associated proteins.

534 Plantae

535 Plants accumulate LDs in both vegetative and reproductive tissues (Chapman et al.
536 2012). The involvement of LDs in the physiology and development of plants are
537 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.

583 Dioxygenases (α -DOX) have also recently been found to be associated with LDs
584 in leaves of senescent *Arabidopsis thaliana* cells (Shimada et al. 2014). *A. thaliana*
585 has two homologs of these dioxygenases (At α -DOX1 and At α -DOX2). At α -DOX1
586 is localized on LDs of leaves and At α -DOX2 is located in the ER (Shimada et al.
587 2014). These enzymes produce an oxylipin (2-HOT) from α -linolenic acid (18:3).
588 These molecules participate in defense mechanisms in response to biotic and abiotic
589 stresses (Shimada and Hara-Nishimura 2015).

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

593 Seeds are the most studied structure for the understanding of OLDs because they
594 are able to accumulate TAGs in the form of LDs reserve to support germination after
595 the end of the dormancy phase (Huang 1996). In special cases such as jojoba seed
596 (*Simmondsia chinensis*), LDs can contain cerides (Yermanos 1975). These LDs are
597 small (between 0.5 and 1.5 μ m) conferring a large surface area per unit of TAGs,
598 facilitating the binding of lipases during germination (Huang and Huang 2015).
599 Numerous proteomic studies have been performed in plant models: *Brassica napus*
600 (Katavic et al. 2006; Jolivet et al. 2009), *Arabidopsis thaliana* (Jolivet et al. 2004;
601 Vermachova et al. 2011), *Sesamum indicum* (Lin et al. 2005), *Jatropha curcas*
602 (Popluechai et al. 2011; Liu et al. 2015), *Madia sativa* (Acevedo et al. 2012),
603 *Gevuina avellana* (Acevedo et al. 2012), *Zea mays* (Tnani et al. 2011), *Camelina*
604 *sativa* (Jolivet et al. 2013), or *Arachis hypogaea* (Jolivet et al. 2013). These
605 proteomic analyses show that LDs are covered with oleosins with a minor presence
606 (less than 5%) of caleosin and steroleosin (Chapman et al. 2012; Murphy 2012).
607 Oleosins are involved in regulating the size and stability of LDs of seeds (Chapman
608 et al. 2012). LDs have also been characterized in the tapetum cells of the anther
609 (Hsieh and Huang 2004) and in pollen grains and pollen tubes (Kretzschmar et al.
610 2018). A focused study on PUX10 (Plant UBX Domain-containing Protein 10)
611 whose localization was confirmed by fusion with enhanced GFP (eGFP) on the
612 surface of LDs during embryonic development, seed germination, and pollen tubes,
613 showed that PUX10 recruits by its UBX domain an AAA-type ATPase Cell Cycle
614 48 (CDC48) that facilitates the transfer of polyubiquitinated protein to the 26S
615 proteasome (Kretzschmar et al. 2018).

616 LDs are also present in *Arabidopsis thaliana* leaves. The number of LDs is very
617 low in healthy leaves. LDs accumulate more in the leaves in the senescence phase
618 (Shimada et al. 2015) with a variable size of 1–18 μ m (Lersten et al. 2006). In
619 particular, the expression of *A. thaliana* caleosin-3 as well as At α -DOX1 increases
620 during senescence (Shimada et al. 2014). Proteomic analysis of the LD of aging
621 leaves of *A. thaliana* was performed: 28 proteins including 9 enzymes involved in
622 the secondary defense metabolism of the plant were identified (Brocard et al. 2017).
623 The analysis also revealed the presence of the Small Rubber Particle 1 (AtSRP1)
624 protein. Functional analysis of AtSRP1 reveals that this protein modulates the
625 expression of caleosin-3 in aging leaves. In addition, overexpression of AtSRP1
626 induces an increase in 18:3 enriched TAG accumulations from galactolipid recycling
627 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 Chlororarachniophytes and (2) the red

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

671 Organisms from the Green Lineage: *Euglenozoa*

672 At least two independent events of secondary endosymbiosis between a green alga
673 and an unknown heterotrophic eukaryotic organism led to the appearance of
674 Euglenozoa and Chlororarachniophytes (Petroutsos et al. 2014; Füssy and Oborník
675 2018). Chlororarachniophytes (*Bigelowiella natans*) contain a four-membrane chlo-
676 roplast and a residual nucleus, called the nucleomorph, located between the two most
677 internal and external membranes of the chloroplast (Petroutsos et al. 2014). To our
678 knowledge, no data are available on LD formation in Chlororarachniophytes.
679 *Euglenozoa* comprises photosynthetic species with a chloroplast bounded by three
680 membranes but also parasitic species devoid of any chloroplast (Petroutsos et al.
681 2014). Several studies report that parasitic organisms such as *Trypanosoma cruzi*,
682 the agent of Chagas disease (D'ávila et al. 2011), are capable of inducing the
683 formation of large LDs in macrophages. In photosynthetic organisms, *Euglena*
684 *gracilis* is a microalga living in freshwater, interesting for the research of alternatives
685 to petroleum resources because it accumulates wax esters inside LDs in nitrogen
686 starvation. These wax esters come from the conversion of a crystalline β -1,3-glucan,
687 paramylon. Wax esters can be used as fuel for aviation but also as biofuel after
688 refining (Guo et al. 2017). To our knowledge, there is no detailed study of the
689 structure of the LD of *E. gracilis*.

690 Organisms from the Red Lineage: *Chromista/Chromalveolates*

691 *Apicomplexa (Containing a Non-photosynthetic Plastid)*

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

701 *Dinophyta*

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

and symbionts of coral-dinoflagellate endosymbiosis (Kellogg and Patton 1983). 705
 The first proteomic analysis of the gastrodermale LDs of *Euphyllia glabrescens* 706
 (*Cnidaria*) revealed the presence of 42 proteins involved in the metabolism of lipids 707
 and proteins as well as in the response to some stresses (Peng et al. 2011). A second 708
 proteomic analysis of LDs of *Symbiodinium* sp. associated with coral tentacles of 709
Euphyllia glabrescens, highlighted a 20 kDa Symbiodinium Lipid Droplet Protein 710
 (SLDP) that plays a role in the structural and functional stability of the LD (Pasaribu 711
 et al. 2014b). Proteomic analysis of LDs of isolated *Symbiodinium* spp. has also been 712
 performed (Jiang et al. 2014). This study did not reveal the presence of SLDP but of 713
 several proteins involved in lipid metabolism (Sterol transfer family protein), cell 714
 signaling (14-3-3 protein, ADP ribolysis factor), stress response (HSP90), and 715
 energy metabolism (ATP synthase F1 subunit α , GTP binding protein). 716

Haptophyta 717

Haptophyta include photosynthetic species forming extracellular shells of calcium 718
 carbonate (coccolithophores' coccospheres) and non-calcified cell walls (Cavalier- 719
 Smith 1986). A proteomic study was carried out in the non-calcareous haptophyta 720
Tisochrysis lutea, formerly *Isochrysis* aff. *galbana* (Bendif et al. 2013). This 721
 microalga is interesting for the aquaculture industry because it is enriched in DHA 722
 (Hubert et al. 2017). It is also known to accumulate a particular neutral lipid in place 723
 of TAGs: an alkenone, a very long chain ketone (C_{37} to C_{40}), within an alkenone 724
 body (AB) (Marlowe et al. 1984a, b). The purification of LDs highlighted the 725
 presence of 74.2% of alkenone (C_{37} and C_{38}), 24.6% of other lipids, and 1.2% of 726
 alkene. Proteomic analysis revealed the presence of 514 proteins on the surface of 727
T. lutea AB, of which three are predominant: a V-ATPase identified previously in a 728
 proteomic analysis in nitrogen deficiency (Song et al. 2013), an SPFH (Stomatin/ 729
 Prohibitin/Flotillin/HflK) domain-containing protein, localized in the endoplasmic 730
 reticulum, associated with a lipid raft and a hypothetical protein (Shi et al. 2015). 731
 With the exception of SPFH, the transition between a rich medium and a low 732
 nitrogen medium causes an increase in the expression of the target genes encoding 733
T. lutea AB proteins (Shi 2019). 734

Heterokonta 735

Heterokonta (or Stramenopiles) constitute a superphylum in the *Chromista* kingdom 736
 (Cavalier-Smith 2018). Five proteomic studies were performed in four model spe- 737
 cies: *Nannochloropsis oceanica* (Vieler et al. 2012), *Phaeodactylum tricoratum* 738
 (Yoneda et al. 2016; Lupette et al. 2019), *Fistulifera solaris* (Nojima et al. 2013), 739
 and *Aurantiochytrium limacinum* (Watanabe et al. 2017). The first study conducted 740
 with *N. oceanica* (Vieler et al. 2012) revealed a 16.8 kDa Lipid Droplet Surface 741
 Protein (LDSP) by proteomic analysis. This protein is present in the six species of 742
Nannochloropsis with functional features close to plant oleosins as well as MLDP of 743
Chlamydomonas reinhardtii. The second proteomic study in diatoms concerns the 744
 pennate diatom *Fistulifera solaris* (Nojima et al. 2013). The authors did not detect 745

746 any major band and the purification seems to involve protein contaminations. By
747 subtracting the protein purification proteins from the soluble fraction, the authors
748 identified fourteen candidate proteins. By searching for conserved domains, one of
749 the identified proteins has a quinoprotein-alcohol dehydrogenase-like domain
750 detected in the proteome of LDs of *Camelina sativa* seed (Jolivet et al. 2013) but
751 has no hydrophobic domain. This protein of 506 amino acids was named DOAP1
752 (Diatom-Oleosome-Associated-Protein 1). Based on a fusion between GFP and the
753 N-terminal signal sequence of DOAP1 (Maeda et al. 2014), this protein was initially
754 present in the ER. The third model characterized is the pennate diatom
755 *Phaeodactylum tricorutum*. A first proteomic study allowed the identification of
756 five major proteins on the surface of the LD of *P. tricorutum*, including the
757 Stramenopile LD Protein (StLDP) (Yoneda et al. 2016). This class I protein was
758 localized to the surface of the LD by fusion with a GFP protein (Yoneda et al. 2018).
759 By optimization of the *P. tricorutum* LD purification protocol, a second study was
760 carried out in this organism (Lupette et al. 2019). The proteome of the LD is
761 composed of 86 proteins including most notably the LD-protein StLDP, metabolic
762 actors, organelle membrane-associated proteins, proteins implicated in the treatment
763 of genetic information, or chaperones involved in protein quality control. The
764 hydrophobic core is only made of TAG surrounded by a monolayer of polar lipids
765 consisting of PC, SQDG, and two molecular species (20:5–16:1 and 20:5–16:2) of
766 diacylglycerylhydroxymethyltrimethyl- β -alanine (DGTA), a betaine lipid. A sterol
767 probably located in the polar lipid monolayer, brassicasterol, was also detected and
768 specific enrichment of β -carotene has been observed (Lupette et al. 2019). A last
769 proteomic study was recently conducted on the model *Aurantiochytrium limacinum*
770 F26-b, a non-photosynthetic unicellular microalga classified as *Heterokonta*. This
771 microalga is particularly studied for its biotechnological potential because it is
772 a species rich in DHA (22:6) (Dellero et al. 2018a, b; Morabito et al. 2019). A
773 proteomic study of the LD fraction of *A. limacinum* F26-b identified a
774 Thraustochytrid-specific Lipid Droplet Protein 1 (TLDP1) (Watanabe et al. 2017).
775 A mutant of *tldp1* shows a decrease in the amount of TAG and the number of lipid
776 droplets per cell. However, larger and irregular LDs are observed in this mutant.
777 TLDP1 may regulate the accumulation of TAGs as well as the size and number of
778 LDs in *A. limacinum* F26-b (Watanabe et al. 2017).

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

11.4 Lipid Droplets Are Dynamic Structures 786

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 793**11.4.1.1 PHA Biosynthesis** 794

PHA biosynthesis routes have been recently reviewed by Mozejko-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 804

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 811**11.4.2.1 Biogenesis of PHA Granules in Prokaryotes** 812

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

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

819 **11.4.2.2 Biogenesis of Cytosolic TAG Droplets in Eukaryotes**

820 The general principles of the biogenesis mechanism seem to be similar in
821 non-photosynthetic and photosynthetic organisms (Fig. 11.2—TAG synthesis).
822 The first step consists of the synthesis of TAG at the level of the ER, through the
823 Kennedy pathway (see Sect. 11.3.1.2). Following the synthesis of TAGs, a “lens” of
824 neutral lipids (TAGs and sterol esters) develops within the bilayer of the ER at
825 particular nucleation sites (Fig. 11.2—Nucleation) (Pol et al. 2014; Wilfling et al.
826 2014). A number of pre-droplets are formed in the ER from freely diffusing TAG
827 molecules, but only a small proportion of them will ultimately form a lipid droplet.
828 The lifespan of these lipid pre-droplets is estimated at a few milliseconds (Khandelia
829 et al. 2010). This phenomenon leads to an energy optimum called a nucleation
830 barrier (Thiam and Forêt 2016). The homogeneity of the phospholipid bilayer of the
831 reticulum is also an important parameter to take into account the success of nucle-
832 ation. Indeed, on a uniform bilayer, the nucleation of LDs could occur randomly.
833 However, the phospholipid bilayer of the ER is heterogeneous in its biochemical
834 composition. There are therefore specific sites where the nucleation energy is lower.
835 Nucleation can be favored by four different phenomena: (1) the curvature of the
836 membrane developing hydrophobic defects favorable to the accumulation of TAG,
837 (2) the synthesis sites of TAGs defined by the presence of DGATs, or other synthetic
838 enzymes such as PDATs, (3) the presence of proteins (SEIPIN, FIT, PLINs, ACSL)
839 inducing curvature in decreasing the bending energy of the membrane, and (4) the
840 presence of proteins and/or lipids interacting with TAGs (Thiam and Forêt 2016).

841 Following the formation of this lens, budding (Fig. 11.2—Budding) of the LD
842 occurs on the cytoplasmic side of the ER (Walther et al. 2017). This polarized
843 budding of LDs follows an unknown mechanism. In humans, it has been proposed
844 that an ER protein, FIT2 (Fat-inducing transcript 2), was involved in the polarization
845 of budding (Choudhary et al. 2016). There are currently several models concerning
846 the growth of the LD, including the coalescence and Ostwald ripening (Thiam et al.
847 2013b). Coalescence is a physical mechanism corresponding to the fusion of two
848 identical substances (in our case, the fusion of two LDs). When an emulsion of oil
849 and water is vigorously mixed, the fusion of LDs with each other is observed to form
850 a giant lipid droplet reflecting the separation between the oil and the water. Ostwald
851 ripening is a destabilization mechanism corresponding to the gradual disappearance
852 of small LDs from an emulsion to give way to larger LDs (Thiam et al. 2013b). This
853 mechanism begins with the transfer of TAG molecules from small LDs to larger
854 ones. The direction of the transfer is dictated by the pressure difference of Laplace
855 (Thiam et al. 2013b; Thiam and Forêt 2016).

856 An increase in the contact angle between the LD and the ER bilayer causes fission
857 of the LD (Fig. 11.2—Fission). This mechanism also involves proteins on the
858 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 859 et al. 2006). SEIPIN is involved functionally in the initial stages of LD formation 860 into generating nascent structures or participating in their maturation in mature LD 861 (Wang et al. 2016). A morphological screen of LDs of *Saccharomyces cerevisiae* 862 shows that the homolog Fld1 of SEIPIN is necessary for the formation of “normal” 863 droplets (Fei et al. 2008). This protein also participates in the stabilization of the 864 contact sites between the ER and the LD (Salo et al. 2016). A recent study also 865 proposes that the SEIPIN protein and the LDAF1 (Lipid Droplet Assembly Factor 1) 866 protein form an oligomeric complex of approximately 600 kDa in the ER bilayer 867 determining the LD formation sites (Chung et al. 2019). SEIPIN is also involved in 868 the regulation of TAG synthesis: it interacts notably with GPAT, AGAT2, and lipins 869 (phosphatidate phosphatases recruited for the synthesis of DAG, serving as the 870 substrate to the production of TAG). A single Seipin gene is detected in animals 871 and fungi studied so far, whereas three homologs (SEIPIN1, 2, and 3) were reported 872 in higher plant models (Taurino et al. 2017). SEIPINS are divided into two mono- 873 phyletic groups in plants according to their degree of functional specialization (Cai 874 et al. 2015). A functional genetic study shows that double and triple mutations cause 875 the accumulation of larger LDs in *Arabidopsis* (Taurino et al. 2017). An ortholog of 876 SEIPIN was also detected in the diatom *Phaeodactylum tricornutum* (Lu et al. 2017). 877 The overexpression of SEIPIN in *P. tricornutum* causes an increase in the size of 878 LDs, the amount of neutral lipids (57% increase), and the proportion of saturated 879 fatty acids (16:0) (Lu et al. 2017). 880

LDs can detach from the ER, forming a population of initial LDs (iLD) measuring 881 400–800 nm in diameter (Walther et al. 2017). These LDs can be subsequently 882 converted, according to an unknown mechanism, into a new population of LDs 883 called expanding lipid droplets (eLDs) (Walther et al. 2017). This conversion 884 requires the acquisition of an independent TAG synthesis machinery carried out 885 by GPAT4 and DGAT2 (Kuerschner et al. 2007; Stone et al. 2009; Wilfling et al. 886 2013). These two proteins initially present in the ER are relocated to the LD 887 (Wilfling et al. 2013). 888

Although the conversion mechanism is unknown, there is still evidence that the 889 vesicular machinery ARF1/COP1 (ADP Ribosylation Factor 1/Coat Proteins type I) 890 is involved in this process. In particular, it has been shown in an in vitro system that 891 the ARF1/COP1 machinery allows the synthesis of nano LDs of 60–80 nm (Thiam 892 et al. 2013a). COP1 is a complex composed of seven subunits (α , β , β' , γ , ϵ , δ , ζ) 893 responsible for the retrograde vesicular transport of the Golgi apparatus to the ER as 894 well as intra-Golgi transport (Beck et al. 2009; Jackson 2014). ARF1 is a member of 895 the family of GTP-binding proteins of the Ras superfamily (small GTPases). ARF1 896 is localized in the Golgi apparatus and was initially shown to act in intra-Golgi 897 transport. The role of the ARF1/COP1 system in LD dynamics needs therefore to be 898 clarified further compared to that in Golgi dynamics. 899

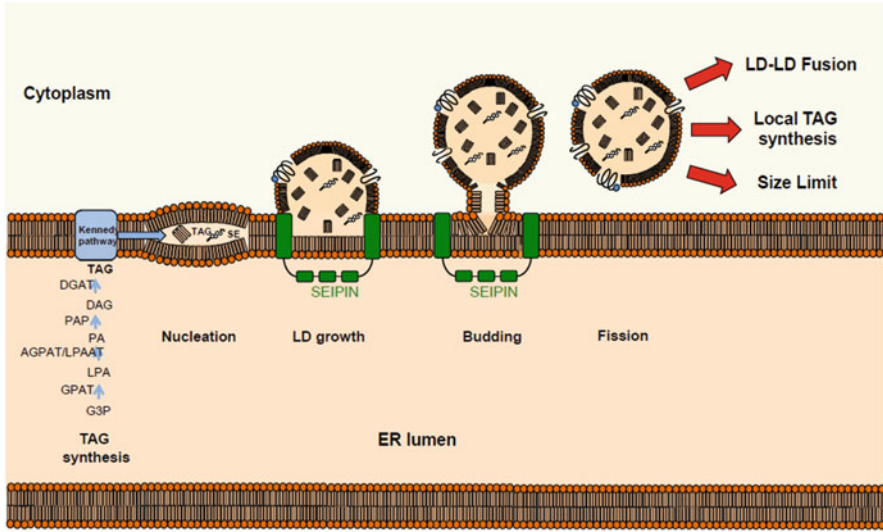


Fig. 11.2 Principles of lipid droplet biogenesis in eukaryotes

900 11.4.3 Catabolism of Lipid Droplets

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

905 11.4.3.1 Degradation of PHA in Prokaryotes

906 The degradation of PHA granules is carried out by a PHA depolymerase (PhaZ)
 907 (Uchino et al. 2008). PhaZ plays a role similar to acyl lipases in eukaryotes (see
 908 below). At least two degradation pathways of PHA granules exist: (1) an extracel-
 909 lular pathway in bacteria capable of secreting depolymerases in the environment and
 910 (2) an intracellular route, less well known to date. Seven intracellular PHB
 911 depolymerases (PhaZ1 to PhaZ7) as well as two PHB hydrolases (PhaY1, PhaY2)
 912 have been described in the *R. eutropha* H16 model (Abe et al. 2005; Uchino et al.
 913 2008; Sznajder and Jendrossek 2014). However, PhaZ1 appears to be the only active
 914 PHB depolymerase in *R. eutropha* H16 (Sznajder and Jendrossek 2014). PhaZ1
 915 presents a cysteine in the catalytic site of the “box” lipase. The thiolysis of PHB
 916 granules into (R)-3-hydroxybutyryl-CoA by PhaZ1 has been demonstrated (Eggers
 917 and Steinbüchel 2013). Localization of PhaZ1 has been confirmed on the surface of
 918 PHB granules by fusion with eYFP (Uchino et al. 2008). A mechanistic model of
 919 PHB granule degradation was proposed by Eggers and Steinbüchel (2013).

11.4.3.2 Degradation of TAG in Eukaryotes 920

Fatty Acid Mobilization 921

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

LDs are strongly reorganized during lipolysis following a process that reduces 947
their size and increases the surface/volume ratio and accessibility to lipases. Two 948
models exist for the formation of these lipid microdroplets: (1) fragmentation of a 949
giant LD (Moore et al. 2005) and (2) capture of released FAs, which are potentially 950
toxic following lipolysis (Paar et al. 2012). A fat-specific-protein of 27 kDa (FSP27 951
also known as CIDEC for cell death-inducing DNA fragmentation factor alpha-like 952
effector c) binds to ATGL at the surface of LDs inhibiting the lipolysis mechanism 953
by blocking access of CGI-58 to ATGL. A reduction in FSP27 expression by 954
siRNAs in a 3T3-L1 mouse line causes the formation of multiple small LDs as 955
well as an increase in lipolytic activity (Nishino et al. 2008). FSP27 proteins are 956
involved in lipid transfer mechanisms between LDs favoring mechanisms of fusion, 957
growth, and enlargement (Gong et al. 2011; Jambunathan et al. 2011). FSP27 is 958
notably in direct interaction with PLIN1a in white adipose tissues favoring the 959
formation of LDs (Grahn et al. 2013; Sun et al. 2013). The size of LDs therefore 960
seems regulated also by a balance between PLIN1a and FSP27. 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 retromeric 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 1010

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 1028

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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