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REVIEW ARTICLE

The dynamic roles of intracellular lipid droplets: from archaea to mammals

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Abstract During the past decade, there has been a paradigm shift in our understanding of the roles of intracellular lipid droplets (LDs). New genetic, biochemical and imaging technologies have underpinned these advances, which are revealing much new information about these dynamic organelles. This review takes a comparative approach by examining recent work on LDs across the whole range of biological organisms from archaea and bacteria, through yeast and *Drosophila* to mammals, including humans. LDs probably evolved originally in microorganisms as temporary stores of excess dietary lipid that was surplus to the immediate requirements of membrane formation/turnover. LDs then acquired roles as long-term carbon stores that enabled organisms to survive episodic lack of nutrients. In multicellular organisms, LDs went on to acquire numerous additional roles including cell- and organism-level lipid homeostasis, protein sequestration, membrane trafficking and signalling. Many pathogens of plants and animals subvert their host LD metabolism as part of their infection process. Finally, malfunctions in LDs and associated proteins are implicated in several degenerative diseases of modern humans, among the most serious of which is the increasingly prevalent constellation of pathologies, such as obesity and insulin resistance, which is associated with metabolic syndrome.

Keywords Lipid droplet · Trafficking · Membrane · Signalling · Storage

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Abbreviations

ABA	Abscisic acid
DAG	Diacylglycerol
ER	Endoplasmic reticulum
LD	Lipid droplet
MLDP	Myocardial lipid droplet protein
PA	Phosphatidic acid
PAT	Perlipin, adipophilin and TIP47 (now termed Plin)
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHB/V	Co-polymer of hydroxybutyrate and hydroxyvalerate
Plin	Perilipin
PPAR	Peroxisome proliferator-activated nuclear receptor
TAG	Triacylglycerol
TIP47	Tail-interacting 47-kDa protein (or Plin3)
WS	Wax ester synthase
WS/DGAT	Wax ester synthetase/diacylglycerol acyltransferase

Introduction

The purpose of this article was to review recent progress in elucidating the origins, organization and functions of intracellular lipid droplets (LDs). Over the past few years, several articles have summarized various aspects of LD-related studies. Examples include: Martin and Parton (2005, 2006), Wältermann and Steinbüchel (2005), Fujimoto and Ohsaki (2006), Wolins et al. (2006a, b), Welte (2007,

2009), Ducharme and Bickel (2008), Fujimoto et al. (2008), Goodman (2008, 2009), Thiele and Spandl (2008), Walther and Farese (2009), Farese and Walther (2009), Guo et al. (2009), Murphy et al. (2009), Ohsaki et al. (2009), Olofsson et al. (2009), Zehmer et al. (2009b), Arrese and Soulages (2010), Beller et al. (2010b), Fujimoto and Parton (2010), Kalantari et al. (2010), Bozza et al. (2011), Kühnlein (2011), Greenberg et al. (2011) and Greenberg and Coleman (2011). Most of these articles focus on specific organisms and/or LD functions, and they are all recommended to readers interested in more detailed accounts of LDs in major groups such as prokaryotes, animals, plants and fungi. The present review is intended to complement these accounts and especially to draw together findings across all biological taxa in order to appreciate more fully both the similarities and differences in LD function in cells and organisms.

Many, and perhaps all, eukaryotic and bacterial cells contain varying amounts of cytosolic lipidic inclusions, and similar structures are also found in several types of archaea. These structures occur most commonly as spheroidal macromolecular assemblies of neutral lipid esters or lipid-based polymers that are normally bounded by a phospholipid monolayer membrane plus a variety of lipid-associated proteins. Cytosolic lipid inclusions in cells were originally described by Hanstein (1880), Altmann (1890), and Wilson (1896) and were originally called microsomes or liposomes. However, these terms were later appropriated by others to describe quite different bilayer-based lipid structures. Depending on the organism and scientific field, cytosolic neutral lipid inclusions have been variously referred to as lipid bodies, lipid droplets, adiposomes, granules, oleosomes, or oil bodies. Despite several attempts to arrive at a common terminology (Murphy 2001), several of the above names are still in widespread use in the literature. Although we originally favoured 'lipid body' (Murphy and Vance 1999; Murphy 2001), by far, the most common term in the current literature is 'lipid droplet' (Martin and Parton 2006). Therefore, this term, abbreviated as LD, is used in the present article.

During most of the twentieth century, intracellular LDs in multicellular organisms were almost universally regarded as specialized storage organelles that were largely limited to specific cell types such as adipocytes and steroidogenic cells in mammals; fat bodies in insects: and cotyledon, mesocarp or scutellar cells in plants. In most cases, these lipid deposits were believed to be relatively long-term carbon stores with slow rates of turnover. During the 1990s, however, evidence began to accumulate that some cytosolic LDs had more dynamic roles, e.g. as readily accessible sources of inflammatory mediators in leukocytes or steroid hormones in steroidogenic cells. In 1999, we attempted the first synthesis of knowledge about LD formation in both plants and animals (Murphy and Vance 1999). This was followed in 2001 by a more detailed review of LDs that stressed their roles as near-ubiquitous and highly dynamic organelles across the full range of biological organisms (Murphy 2001).

Over the past decade, technical advances in cell biology and the progress in genomics and proteomics have underpinned considerable advances in our understanding of the nature and function of intracellular LDs. The advent of cheap and rapid sequencing of the genomes of whole organisms and transcriptomes of specific cell types, coupled with the increasing ease of generating gene knockout or overexpression lines, has greatly extended or knowledge of LD composition and function. New developments in mass spectrometry have also enabled researchers to monitor lipid remodelling in living cells (de Kroon 2007).

However, some of the most important advances have come from the use of new imaging technologies. These have contributed greatly to our understanding of the real-time dynamics of LD behaviour in cells and in following such behaviour during processes such as inflammatory responses (Melo et al. 2011). Examples of such imaging techniques include: novel fixation methods for LDs in immunofluorescence microscopy DiDonato and Brasaemle 2003); vibrational imaging of LDs in live fibroblast cells (Nan et al. 2003); ^{1}H NMR-visible lipid labels (Wright et al. 2003; Delikatny et al. 2011); third-harmonic generation microscopy (Débarre et al. 2005); fluorescent imaging (Kuerschner et al. 2008); multiplex CARS microscopy (Müller and Schins 2002; Rinia et al. 2008); quantitative electron microscopy (Cheng et al. 2009); freeze-fracture replica immunogold labelling (Robenek et al. 2011); stimulated Raman scattering microscopy (Bewersdorf et al. 2011); and confocal reflection microscopy (Gaspar and Szabad 2009). In the past few years, there have been particular advances in the use of live imaging systems including live microscopy (Digel et al. 2010; Somwar et al. 2011); vital staining in combination with fluorescence-activated cell sorting (Cooper et al. 2010); and time-lapse adaptive harmonic generation microscopy (Watanabe et al. 2010). The dynamic motion of cytosolic LDs as captured by live imaging has been reviewed by Welte (2009).

Studies employing these and other methods have now firmly established the roles of LDs in fundamental cellular processes such as the trafficking of lipids, proteins and entire membranes. Moreover, malfunctions in LDs are implicated in numerous human degenerative conditions including type 2 diabetes, Parkinson's disease, some cancers and Alzheimer's. LDs also play important roles in a number of serious infections including hepatitis, leprosy, Chlamydia, trypanosomiasis, Chagas disease and dengue fever. More recently, LDs have also been exploited for a wide range of biotechnological purposes including as

carriers of pharmaceutical products (Bhatla et al. 2010; Bonsegna et al. 2011) or as renewable, carbon-neutral feedstocks for the manufacture of biodegradable polymers (Anderson and Dawes 1990; Grage et al. 2009; Quillaguamán et al. 2010) and biofuels (Kalscheuer et al. 2007a, b; Beopoulos et al. 2009; Kosa and Ragauskas 2011). In this review, a comparative approach will be adopted to survey recent findings about the nature, roles and exploitation of LDs across each of the major biological domains. The principal emphasis will be on the function rather than on the structures of LDs and their associated proteins. There have been several recent accounts of the structure and targeting mechanisms of LD proteins (Hickenbottom et al. 2004; Zehmer et al. 2008).

Prokaryotes

The cells of all biological organisms are bounded by lipid-based membranes that, except for some archaeal species, are based on lipid bilayer structures (Vereb et al. 2003). It is therefore interesting to pose the question: are all cells also capable of accumulating intracellular lipidic assemblies, and specifically cytosolic LDs? Prokaryotes are divided into bacteria and archaea, but which of these groups has the more ancient lineage is yet to be fully resolved. It is appealing to consider that early cells resembled modern archaea, many of which retain an ability to live in the kinds of extreme environments found on earth when life first evolved well over three billion years ago (Stetter 2006; Berg et al. 2010; Jarrell et al. 2011). However, this hypothesis has been challenged by suggestions that archaea may have evolved more recently and that bacteria are the more ancient taxon (Cavalier-Smith 2006). Archaeal membrane lipids are very different from those of bacteria and eukaryotes as they are made up of glycerol ether lipids rather than glycerol esters. Moreover, archaea do not synthesize fatty acyl esters, which are the most common constituents of LDs; instead, their lipids are based on isoprenoid chains. Despite these differences, however, recent evidence suggests that most bacteria and some (but not all) archaea can form various types of LDs (containing fluid acyl esters) or granules (containing semi-solid lipopolymers).

This begs the question: 'when did intracellular lipid assemblies first arise?' Despite the fundamental differences in their lipid compositions, many archaea and bacteria are able to accumulate LDs or granules of some description (Han et al. 2009; Jendrossek 2009; Rehm and Steinbüchel 1999). Unlike eukaryotes, however, only a minority of prokaryotes can accumulate triacylglycerol (TAG)-rich and/or wax ester-rich droplets. Instead, most lipid-accumulating bacterial and archaeal genera synthesize a range of polymeric lipids, of which the most common are polyhydroxyalkanoates (PHAs), such as polyhydroxybutyrate (PHB) or polyhydroxyvalerate (PHV). Other polymers synthesized by bacteria include polythioesters, which are sulphur analogs of PHAs (Lütke-Eversloh et al. 2001a, b; Tessmer et al. 2007). To date, all known LD-containing archaeal species accumulate PHAs as their exclusive storage lipid components, and current evidence suggests that the ability to accumulate TAG or wax esters has only arisen in bacterial lineages.

The accumulation of LDs or granules in prokaryotes is normally a facultative response to nutrient depletion. In cases where the organism is adapted for nutrient-rich habitats, little or no lipid accumulation is found. Examples of non-lipid-accumulating prokaryotes include lactobacilli, Enterobacteriaceae and methanogenic archaea (Wältermann and Steinbüchel 2005). Unlike in eukaryotes, LDs/granules in prokaryotes appear to act exclusively as energy stores, with the hyper-accumulation of lipid normally occurring in response to specific forms of nutrient limitation, most commonly a low carbon/nitrogen (C/N) ratio. Therefore, the accumulation of lipidic droplets in prokaryotes frequently marks the cessation of growth and division and the entry of cells into a quiescent phase.

PHA accumulators

The most common class of intracellular lipid accumulated by prokaryotes is the PHAs. The monomers that make up PHAs are synthesized from acetyl-CoA via a short pathway, as shown in Fig. 1. For example, the assembly of PHB from acetyl-CoA involves three enzymes respectively encoded by the *phaA*, *phaB* and *phaC* genes, which in most bacteria are located in a single operon (Legat et al. 2010). The most important enzyme in this pathway is the PHA synthase that assembles monomers such as hydroxybutyrate or hydroxyvalerate into either homopolymers, such as PHB, or copolymers, such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB/V). A wide range of archaeal and bacterial taxa can accumulate PHAs via enzymes encoded by distinct, but clearly homologous, sets of *phaA*, *phaB* and *phaC* genes (Han et al. 2009, 2010a, b). For example, at least 15 genera of the salt-tolerant Haloarchaea can accumulate PHAs via variants of the type III PHA synthase found in bacteria (Quillaguamán et al. 2006, 2008; Legat et al. 2010; Han et al. 2010a, b). However, no archaeal taxa have so far been found to contain type I, II or IV *phaC* genes.

The close similarity of archaeal and bacterial type III PHA synthase genes (Baliga et al. 2004; Bolhuis et al. 2006; Han et al. 2007b, 2010a; Lu et al. 2008; Quillaguamán et al. 2010) and the lack of other PHA gene types in archaea suggest that archaeal PHAs originated from the horizontal transfer of an ancestral type III gene from a bacterium (Kalia et al. 2007). Such a suggestion is in line with findings that modern archaea with non-extreme lifestyles have many other genes of diverse

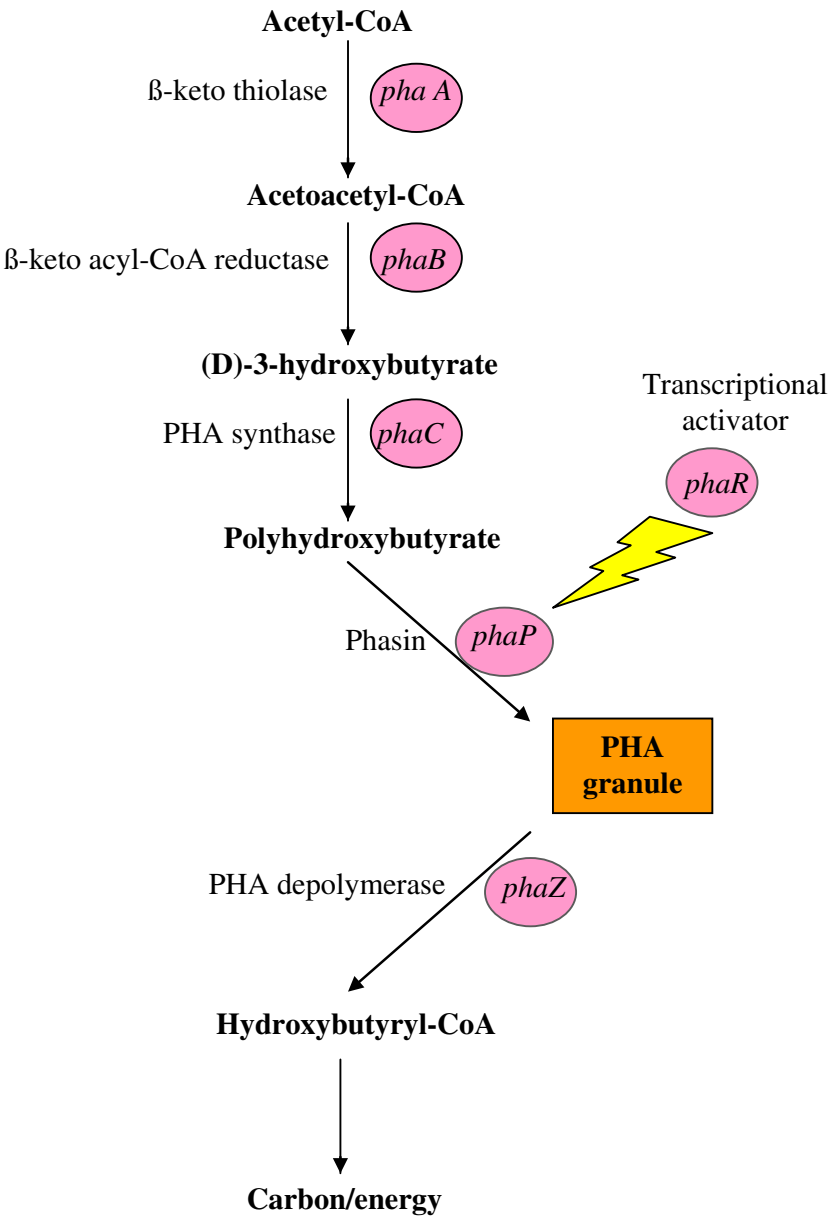

Fig. 1 Polyhydroxyalkanoate metabolism in prokaryotes. In several groups of bacteria and archaea, PHAs can be synthesized via ß-oxidation from acyl substrates such as fatty acids or can be formed de novo from elongation of acetyl-CoA. Fatty acid-derived ß-hydroxyalkanoate monomers have chain lengths of C6 to C16, whilst monomers derived from acetyl-CoA tend to be much shorter, i.e. C4 to C5. Sequence similarity between bacterial and archaeal PHA synthases suggests that achaea acquired the ability to form PHA granules following horizontal transfer of a bacterial *phaC* gene and recruitment of an endogenous phasin analog (see text for further details)

bacterial origins, indicating extensive horizontal acquisition of genes from bacteria over the course of their evolution (Galagan et al. 2002; Koonin 2003). During this time, the original bacterial type III PHA synthase gene has diverged into the distinctive type IIIA PHA synthase variants found in many extant archaea (Kalia et al. 2007; Quillaguamán et al. 2010). The date of archaeal acquisition of PHA synthase genes is unknown, but it has been suggested that it predated the Permian Era and hence occurred over 300 Mya (Legat et al. 2010). However, given that both groups of organisms have been around for over three billion years, the archaeal acquisition of PHA genes may be considerably more ancient.

The mechanism of PHA-accumulation in prokaryotes is of considerable interest in view of its potential biotechnological exploitation to produce biodegradable polymers as industrial feedstocks. Such polymers have biocompatible and thermoplastic features that make them suitable for use in medical implants and as substitutes for petrochemical-derived plastics (Grage et al. 2009; Quillaguamán et al. 2010). Large-scale culture of bioplastic-producing bacterial species, such as *Ralstonia eutropha* (also known in the literature as *Alcaligenes eutropha*, *Wautersia eutropha* or *Cupriavidus necator*), has been attempted, but high costs have meant that such products have so far only found small niche markets (Steinbüchel and Füchtenbusch 1998), although some progress in wider commercial manufacture has been made more recently (Kim and Dale 2005; Dias et al. 2006).

As an alternative to culturing PHA-producing bacteria, the transfer of PHA-related genes to annual or perennial

crop plants could potentially result in larger scale bioplastic production at a significantly lower cost (Slater et al. 1999; Snell and Peoples 2002; Dalton et al. 2011). However, even here, several significant technical hurdles remain, one of the most challenging of which is the cost-efficient extraction of PHA granules from plant tissues such as leaves or seeds (Mooney 2009; Murphy 2010). Another promising approach is to develop archaeal species as industrial-scale PHA producers. In particular, several halophilic archaea have the advantages of utilizing much cheaper carbon sources (including waste materials), as well as having less strict sterilization requirements, plus easier and more efficient methods for PHA extraction (Lillo and Rodriguez-Valera 1990; Koller et al. 2007; Lu et al. 2008; Hezayen et al. 2009).

The number and the size of PHB granules in cells vary according to species and environmental conditions. Accumulation of PHAs in *R. eutropha* results in the formation of approximately 10 to 20 spheroidal cytosolic granules per cell, with diameters ranging from 240 to 500 nm that can amount to as much as 90% of the total cell dry weight (Anderson and Dawes 1990). In contrast, *Azotobacter vinelandii* can accumulate >40 granules per cell, with sizes of 500–1400 nm (Page et al. 1995). A typical 500-nm granule of PHA contains about 40,000 polymer molecules, each of which is made up of about 30,000 PHA monomers. These form a semi-solid lipidic core that is surrounded by a phospholipid monolayer into which several specific granule-binding proteins are embedded.

The major granule-binding proteins include two enzymes of PHA metabolism, namely PHA synthase (PhaC) and PHA depolymerase (PhaZ), a transcriptional repressor (PhaR) and at least four different low-molecular-weight structural proteins called phasins (PhaP). The expression of the major phasin gene, *phaP1*, is regulated by PhaR, which binds to its promoter and represses transcription. During permissive conditions for PHA accumulation, PhaR becomes bound to PHA granules, resulting in a reduced titre of free PhaR and the de-repression of *phaP1* gene expression (Pötter et al. 2002, 2004). Genes encoding *phaP1* homologs have so far only been detected in the ß-proteobacteria, although other proteins bound to PHA granules have also been found in different branches of the proteobacteria and in Gram-positive bacteria (Fukui et al. 2001; Vazquez et al. 1996). PHA granules are mobilized via the PHA depolymerase located on their surface (Kobayashi et al. 2003; Uchino et al. 2008), which is analogous to the roles of acyl lipases in eukaryotic LDs, as discussed below.

Phasins are by far the most abundant proteins on PHA granules and can form as much as 5% of the total cellular protein (Schultheiss et al. 2005). Phasins are non-catalytic structural proteins consisting of a granule-associated hydrophobic domain and a more polar cytosol-exposed domain that stabilize PHA granules and prevent their coalescence (Grage et al. 2009). Although all phasins characterized to date are small amphipathic proteins of 11–25 kDa, phasins from different bacterial genera have no sequence homology and appear to be phylogenetically unrelated (Hanley et al. 1999). This suggests that phasins are a diverse group of small polypeptides that, thanks to their amphipathic properties, have been recruited to serve as structural barriers around PHA granules on multiple occasions during bacterial evolution. The overexpression of endogenous phasin genes in *R. eutropha* resulted in the formation of many small PHA granules, whilst deletion of the phasin gene led to the accumulation of a single large granule of <2,000 nm (Tessmer et al. 2007). Expression of the *R. eutropha* H16 phasin PhaP1 in *Rhodococcus opacus* PD630 and *Mycobacterium smegmatis* mc2155 led to its targeting to TAG-rich LDs where the phasin also acted as an anchor to bind other proteins (Hänisch et al. 2006).

Phasins appear to be restricted to PHA-accumulating bacteria and are not present in any archaeal genome sequenced to date. However, it is possible that proteins with different annotations could carry out phasin-like roles in archaea. For example, in recombinant *Escherichia coli* (which cannot normally make PHAs) engineered to synthesize PHAs, but not expressing phasin genes, large amounts of the endogenous 16-kDa heat-shock protein, HspA, were formed and attached to PHA granules (Tessmer et al. 2007). This enabled recombinant *E. coli* cells to accumulate numerous small, stable PHA granules. However, when the phasin genes of *R. eutropha* (which can normally make PHAs) were deleted, there was no compensatory upregulation of Hsps and the overall amount of PHAs was severely reduced. These data demonstrate that in some species, but not all, HspA can functionally replace phasins as a stabilizer of PHA granules. One could speculate therefore that heat shock proteins or other structurally suitable small polypeptides might stabilize PHA granules in archaea in the absence of phasins. If archaea were to use such ad hoc proteins to stabilize their PHA granules, it may explain why there is such wide variation in PHB granule number and size, for example in some *Halomonas* spp. (Martinez-Canovas et al. 2004; Quesada et al. 2004). Whilst *Halomonas boliviensis* typically synthesizes one or two granules of 200–640 nm per cell, *Halococcus morrhuae* and *Halococcus salifodinae* make several smaller granules of 50–300 nm (Legat et al. 2010).

TAG/wax ester accumulators

Although the majority of bacteria, and many archaea, store carbon in the form of PHAs, a subset of bacteria, primarily

nocardioform actinomycetes, streptomyces and some Gram-negative strains, are capable of storing carbon as LDs enriched in TAGs (Alvarez and Steinbüchel 2002; Wältermann and Steinbüchel 2005). The highest levels of TAG accumulation have been reported mainly in nocardioforms such as the genera *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Micromonospora*, *Dietzia* and *Gordonia* and in some streptomycetes (Kosa and Ragauskas 2011). For example, in the well-studied Gram-positive, non-spore-forming actinomycete, *R. opacus* PD630, grown in low C/N media, LDs of 50- to 400-nm diameter may accumulate to form more than 75% of cellular dry weight (Alvarez et al. 1996), with a potential daily TAG production from organic wastes of almost 60 mg l^{-1} (Gouda 2008). Spherical wax ester-rich droplets of about 200-nm diameter have been reported in some *Acinetobacter* spp., although other species accumulated rectangular or rod-shaped wax ester structures (Wältermann and Steinbüchel 2005). Several TAG-accumulating cyanobacteria, including *Dunaliella salina* and *Synechocystis* spp, are currently being assessed for their potential to act as solar-powered sources of renewable hydrocarbons, especially in the context of the so-called third-generation biofuels (Murphy 2008; Sheehan 2009; Stephens et al. 2010).

Several phylogenetically related marine γ-proteobacteria can utilize petroleum hydrocarbons as energy sources and are therefore of great interest for their potential in dealing with oil spills (Harayama et al. 1999, 2004). Examples of these so-called hydrocarbonoclastic bacteria include the genera *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Neptunomonas*, *Oleiphilus*, *Oleispira* and *Thalassolituus* (Kalscheuer et al. 2007a, b; Steinbüchel 2007). Whilst only present in low abundance in unpolluted water, species such as *Alcanivorax borkumensis* can multiply rapidly in oil-polluted water where they can eventually make up 80–90% of the entire microbial community by mass (Harayama et al. 1999; Kasai et al. 2002). However, this rapid population growth is followed by an equally dramatic crash once the hydrocarbons are used up. During the extended periods when suitable growth substrates are unavailable, hydrocarbonoclastic bacteria enter a dormant phase where they live off their accumulated LD reserves that consist of mixtures of TAGs and wax esters (WE; Kalscheuer et al. 2007a, b).

The biosynthesis of WE and/or TAG is catalyzed by a plasma membrane-associated multifunctional wax ester synthetase/diacylglycerol acyltransferase (WS/DGAT) that is found in many bacterial species (Kalscheuer 2010; Manilla-Pérez et al. 2010; Wältermann et al. 2005). The amino acid sequence of this bacterial enzyme is unrelated to that of any previously identified WS or DGAT in animals, fungi or plants (Kalscheuer and Steinbüchel 2003), which is consistent with its origin after the divergence of prokaryotic and eukaryotic lineages. Microscopic and biophysical evidence suggests that each WS/DGAT enzyme might give rise to a single microdroplet of about 60-nm diameter (Wältermann et al. 2005). These droplets may then coalesce into mature droplets of 300 nm in a process regulated by the protein, TadA. In support of this model, TadA-deficient cells accumulated 35% less TAG than wild-type cells, and TadA-overexpressing cells accumulated very large LDs. There are suggestions that there may be a further, as yet uncharacterized, WS/DGAT-independent TAG biosynthesis pathway in some bacteria. This follows observations that following a double knockout of WS/DGAT genes in *A. borkumensis*, cells were still capable of substantial TAG formation in LDs (Kalscheuer et al. 2007a, b).

It has been difficult to characterize genuine LD-associated proteins in TAG- or wax ester-accumulating prokaryotes due to the prevalence of nonspecific protein binding to LDs during their isolation. However, the product of the *tadA* (TAG accumulation-deficient) gene of *R. opacus* PD630 has recently been shown to be a droplet-associated protein (MacEachran et al. 2010). The TadA protein sequence is similar to the heparin-binding hemagglutinin (HbhA) family from the genus *Mycobacterium*. In the absence of *tadA*, TAG accumulation was decreased by 30-40%, whilst TadA in vitro was able to both bind heparin and to aggregate LDs. Therefore, TadA is hypothesized to mediate the aggregation of the tiny lipid microdroplets that bud off the plasma membrane and eventually coalesce to form larger mature cytosolic LDs. Prokaryotic cells tend to accumulate either PHAs or TAGs as their major lipidic energy store. However, a few actinomycetes such as *Rhodococcus ruber*, are capable of simultaneously synthesizing and accumulating similar amounts of TAGs and the co-polyester, PHB/V, from unrelated carbon sources such as glucose (Wältermann and Steinbüchel 2005).

The formation of LDs and the accumulation of TAGs are now known to play important roles in the metabolism of several pathogenic bacteria, such as *Mycobacterium tuberculosis* and *Mycobacterium bovis* (Garton et al. 2002; Daniel et al. 2004; D'Avila et al. 2006, 2007, 2008). In *M. tuberculosis* and *Mycobacterium leprae*, the TadA homolog, HbhA, acts as a virulence factor that promotes the spread of the pathogen during the early stages of infection (Pethe et al. 2001; de Lima et al. 2009). The pathogenesis of *M. leprae*, the causative agent of leprosy, is also linked to the proliferation of LDs enriched in eicosanoid precursors in a process involving Toll-like receptor organelles (Mattos et al. 2010). Interestingly, *M. tuberculosis* has been shown to accumulate TAG and store it in conspicuous inclusion bodies, similar to those seen for *Rhodococcus* (Garton et al. 2002, Garton et al. 2008; Deb et al. 2009). It is possible that much like TadA in *Rhodococcus*, HbhA in mycobacteria facilitates LD formation and maturation in addition to its predicted role in cytoadherence and

dissemination. Once it is present in its host and encounters an immune response, *M. tuberculosis* begins to accumulate LDs following the induction of TAG synthase genes (Daniel et al. 2004). This enables the bacterium to enter a non-replicative, drug-resistant, dormancy-like state within the body of the host that can last for decades, during which time the now cryptic pathogen survives on its TAG reserves. The often crucial roles of LDs in several mammalian host–pathogen interactions are discussed further in "Mammals".

Plants

All major groups of plants, from unicellular algae to the most complex angiosperms, are able to produce cytosolic LDs in at least some of their cells/tissues. As with animals, plant LDs used to be regarded as relatively inert carbon stores. In higher plants, the ability to accumulate cytosolic LDs was originally thought to be confined to specific tissues, such as oleogenic seeds or fruits. However, evidence is now emerging that demonstrates the presence of dynamic LDs in tissues that do not accumulate long-term lipid stores. In most cases, cytosolic LDs in plants accumulate TAGs, although at least one oilseed species, jojoba or *Simmondsia chinensis*, accumulates fluid wax esters instead (Ohlrogge et al. 1978). In addition to cytosolic LDs, some plant cells accumulate LD-like structures called plastoglobules in their plastid organelles. The lipidic phase of plastoglobules can include TAGs, sterol esters and various lipophilic pigments such as carotenoids. Although there have been fewer studies into plant LDs compared with their non-photosynthetic counterparts, a somewhat similar picture is emerging with regard to the mechanism of LD formation on specific domains of cytosolic endoplasmic reticulum (ER) or plastidial thylakoid membranes and the roles of LDs in several aspects of plant development and function. Some key aspects of the regulation of plant LD formation are summarized in Fig. 2.

Algae

Algae range from simple unicellular organisms to comparatively complex multicellular species such as seaweeds. As with many other unicellular organisms, some algal species accumulate large numbers of cytosolic TAG-rich LDs as storage reserves in response to certain forms of nutrient limitation or abiotic stress (Murphy 2001; Wang et al. 2009). In some cases, these LDs can make up as much as 86% of cell dry weight. Oleogenic marine microalgae are of considerable biotechnological interest both for their ability to synthesize large amounts of high-value lipids and for their possible use as feedstocks for the production of renewable biofuels (Courchesne et al. 2009). Commercially useful lipids accumulated on algal cytosolic LDs or plastoglobules include long-chain polyunsaturates such as docosahexaenoic acid or pigments such as astaxanthin (Liu and Lin 2001). Other novel lipids include very long-chain polyunsaturated alkenones, alkenoates and alkenes (Eltgroth et al. 2005).

Even under normal growth conditions, smaller amounts of LD TAG may function as intermediates in membrane lipid biosynthesis, and perhaps as short-term stores of acyl chains that enable algae to respond to environmental changes that might require the rapid formation of additional membranes. In some cases, algal cells do not accumulate cytosolic LDs, but instead form plastidial lipid deposits termed plastoglobules. Plastoglobules are probably functionally equivalent to cytosolic LDs, but differ in three major respects. First, they are confined to the stroma, which is the major aqueous phase of plastid organelles; second, they can assume several different forms including rods, fibres and globules; and third, they are bounded by a specific family of proteins, variously termed plastoglobulins, plastid lipid-associated proteins and fibrillins. There is a more detailed discussion about these proteins in the section on higher plant plastoglobules below.

The elucidation of algal LDs and plastoglobules has benefited particularly from recent advances in cell biology and genomics. For example, BODIPY 505/515, a green lipophilic fluorescent dye, has been used as a vital stain for LDs in a wide range of live algal cells (Cooper et al. 2010). In addition, the analysis of several genome sequences has helped considerably to fill in gaps in our understanding of algal lipid metabolism (Khozin-Goldberg and Cohen 2011). All algae and terrestrial plants contain plastids, which are the major sites of de novo fatty acid biosynthesis. Newly synthesized saturated or monounsaturated fatty acids must be exported from plastids to the ER for subsequent desaturation, often followed by their re-import to form the photosynthetic membranes. It now seems unlikely that any part of this extensive constitutive trafficking in acyl lipids involves either plastoglobules or cytosolic LDs (Benning et al. 2006). Instead, there appears to be a combination of transporter proteins and vesicular mechanisms acting both in the cytosol and plastids that enables the formation and modification of membrane lipids (von Wettstein 2001: Andersson and Sandelius 2004).

Although algae often accumulate cytosolic LDs, the major class of exclusively LD-associated protein found in plants, namely oleosins, is only found in multicellular terrestrial plants. This implies that, as discussed in detail below, oleosins are not essential for LD formation per se, but may be part of the adaptive response of those plants that have colonized the land (Huang et al. 2009). In contrast to their lack of oleosins, however, algal genomes do encode

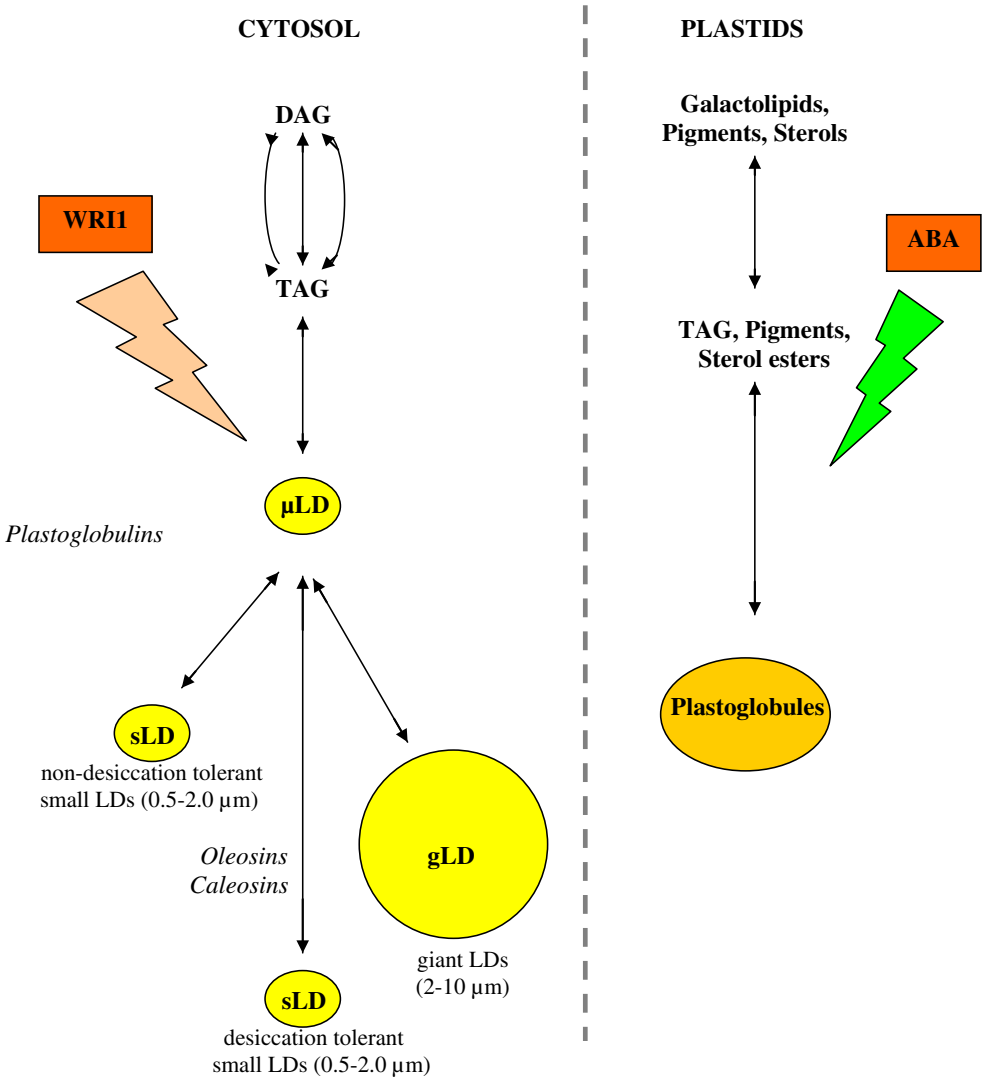

Fig. 2 Lipid droplet regulation in plants. Cytosolic LDs in plants are ER-derived with a TAG core often stabilized by an annulus of LD-binding proteins such as oleosins and caleosins. The presence or absence of these proteins affects the size and function of mature LDs. TAG accumulation in LDs is regulated by master switches such as the transcription factor, WRI1. Plastidial LDs (plastoglobules) may contain a variety of neutral lipids and often maintain intimate contact with the thylakoid membranes from which they are derived. The formation and turnover of plastoglobules are regulated by several physiological effectors, among the most important of which is the hormone, ABA

another putative LD-associated protein, namely caleosins (Partridge and Murphy 2009). At present, it is unclear whether the products of the caleosin-like genes in algae such as *Chlamydomonas reinhardtii* and *Auxenochlorella protothecoides* are true LD-binding proteins or instead act as stress-inducible, membrane-bound enzymes as in some higher plants (see below). Interestingly, orthologs of algal and land plant caleosin genes have been found in many fungal genomes, whereas no such genes are present in any metazoan lineages. This implies that caleosin genes may have been transferred between basal algal and fungal species at some point in the remote past (Partridge and Murphy 2009).

A promising candidate for an authentic LD-associated structural protein in algae is the recently reported major lipid droplet protein (MLDP), a relatively hydrophobic 27-kDa polypeptide that was the most abundant constituent of the proteome of *C. reinhardtii* LDs (Moellering and Benning 2010), plus a possible homolog from *Haematococcus pluvialis* (Peled et al. 2011). Additional members of the *C. reinhardtii* LD proteome include predicted lipid metabolism enzymes and orthologs of various proteins also found associated with animal LD proteomes. Examples include predicted components of vesicular trafficking pathways, such as subunits of the COPI complex and its putative regulator, ARF1a, as well as other small Rab-type GTPases. This implies that the cytosolic LDs of even a simple unicellular alga like *C. reinhardtii* may have similar functional properties to the LDs of relatively complex metazoans such as *Drosophila* and mammals. The partial RNAi-mediated downregulation of MLDP (by <60%) resulted in an increase of about 40% in LD diameter, which is consistent with a structural role that may be analogous to that of perlipin, adipophilin and TIP47 (PAT)/Perilipin proteins in animals and the oleosins in land plants (Moellering and Benning 2010). Although possible orthologs of MLDP are present in other green algae such as *Chlorella vulgaris* and *Volvox carteri*, no orthologs were found in the genomes of diatoms, red algae or even in the green algal *Ostreococcus* spp. This implies that MLDP has

arisen only in some green algal groups and was either always absent from or was subsequently lost from the lineage that produced land plants.

Land plants

The accumulation of cytosolic LDs in oleogenic tissues of terrestrial plants has been comprehensively reviewed previously (Murphy 2001, 2005; Herman 2008; He and Wu 2009; Baud and Lepiniec 2010), as has the accumulation of plastidial LDs, or plastoglobules, in many tissues (Bréhélin et al. 2007). Cytosolic TAG-rich LDs have also been detected in the leaf mesophyll cells of many angiosperms, although much of this literature is rarely cited, as discussed by Lersten et al. (2006). More recently, the case for leaves as sites of LD accumulation has been strengthened considerably. For example, Slocombe et al. (2009) noted that constitutive levels of TAG accumulation in leaves could be increased 10- to 20-fold following manipulation of fatty acid breakdown and lipid synthesis pathways. This indicates that, as in many other organisms, cytosolic LDs in leaves can act as a buffer to take up and/or release acyl moieties in order to maintain the optimal levels of these potentially disruptive metabolites in cells. Progress has also been made, thanks to technological advances such as quantitative electron microscopy (Neuberger et al. 2008), direct organelle mass spectrometry (Horn et al. 2011), and recombinant methods such as the creation of poly-oleosin fusion proteins (Scott et al. 2010) and the selective knockout of different oleosin isoforms (Schmidt and Herman 2009; Wu et al. 2010).

The physical mechanism of cytosolic LD formation in plant cells appears to be very similar to that of animals, namely a localized accumulation of TAG in specialized ER microdomains followed by the release of small LDs that mature to larger droplets under the control of specific proteins (Gidda et al. 2011). Other aspects of the roles of the ER as a multi-domain organelle in plants have been reviewed by Sparkes et al. (2009). There is probably a dynamic two-way flow of lipids between the ER and LDs in most plant cells whereby small numbers of LDs are constantly being formed and then recycled back to the ER. In order to increase the accumulation of LDs, their recycling must be prevented, and this may be one of the roles of the highly abundant LD-binding proteins such as oleosin, and possibly caleosin. When oleosin production in developing soybean seeds was suppressed using RNAi, the formation of LDs was severely disrupted (Schmidt and Herman 2009). Instead of producing normal mature LDs of about 1-μm diameter, many small 50-nm LDs were formed, some of which were recycled to the ER whilst others fused to create giant irregular LDs. The presence of similar giant LDs was also observed in an insertion mutant of *Arabidopsis* (Rodrigo et al. 2006). In both cases, reduction of oleosin formation led to cellular damage and decreased seed viability.

The accumulation of LDs in plant cells is regulated at the transcriptional level by a hierarchy of transcription factor proteins, of which one of the most important is WRINKLED1 (WRI1), a member of the APETALA2/ETHYLENE-RESPONSIVE ELEMENT BINDING (AP2/EREB) family (Cernac and Benning 2004; Cernac et al. 2006; Sanjaya et al. 2011). Several recent studies have shown that WRI1 is one of the key master switches that lead to TAG and cytosolic LD accumulation in higher plants (Baud et al. 2007, 2009; Maeo et al. 2009; Baud and Lepiniec 2010; Pouvreau et al. 2011; Tranbarger et al. 2011). Ectopic expression of WRI1 in tissues that do not normally accumulate large amounts of LDs, such as leaves, leads to the formation of numerous TAG-rich oleosin-bound LDs in a manner that is normally seen only in seeds (Liu et al. 2010; Shen et al. 2010).

It is now emerging that cytosolic LDs in many, but not all, plant cells are bounded by oleosins. Oleosins have an unusually hydrophobic central domain that mediates binding to LDs (Gohon et al. 2011; Li et al. 2002). Oleosins are the major protein associated with LDs in desiccation-tolerant seeds. In those mainly tropical oilseeds that do not undergo desiccation as a normal part of maturation, oleosins are much less abundant (Guilloteau et al. 2003), or may be absent (Murphy 2001). There are several reports that suggest roles for oleosins in the stabilization of LDs in relation to desiccation and freezing tolerance (Leprince et al. 1998; Shimada et al. 2008; Shimada and Nara-Nishimura 2010). However, even the presence of oleosins did not prevent freezing-induced LD fusion and consequent cell disruption in *Cuphea* spp. seeds enriched in TAGs containing crystallization-prone saturated acyl residues (Crane et al. 2003, 2006; Volk et al. 2006). It has been reported that oleosins are present in LD-enriched gametophytes and spores of the moss, *Physcomitrella patens*, but are absent in algae (Huang et al. 2009). This is consistent with the acquisition of oleosins by terrestrial plants as part of their adaptation to life on dry land. All the cytosolic LDs in *P. patens* cells appear to maintain physical continuity with the ER, whereas the LDs in the seeds of higher plants lose their connection with the ER following dehydration. The role of oleosins in preventing LD fusion is supported by the finding that oleosin-rich LDs in embryo, aleurone and scutellum cells of oat grains remained small, whereas oleosin-poor LDs in the endosperm cells of the same grains underwent fusion to create much larger structures (Heneen et al. 2008).

Recent findings suggest that the regulation of LD turnover in plants may have significant similarities with comparable processes in animals. As discussed in "Mammals", Chanarin–Dorfman syndrome in humans is a neutral lipid disorder

characterized by the hyper-accumulation of LDs in ectopic locations. This disease is caused by a malfunction in an α/ß-hydrolase-5 (also called CGI-58) that reduces the ability of cells to mobilize TAG in LDs. Disruption of a lipase-encoding CGI-58 homolog in *Arabidopsis* led to a similar pattern of LD accumulation in ectopic locations such as leaves (James et al. 2010). These findings are consistent with the notion that many, and maybe all, plant cells are able to accumulate small amounts of TAG in cytosolic LDs as part of constitutive acyl lipid trafficking (Murphy 2001; Wahlroos et al. 2003), and possibly as part of various stress responses (Coca and Segundo 2010). The small number of these rapidly turning over LDs may render them difficult to detect in normal non-lipid-accumulating cells. However, when their mobilization is blocked by a malfunctioning CGI-58-like lipase, LDs accumulate to relatively high levels. Similarly to yeast and mammal models, plants appear to have several lipin homologs, *pah*, that play important roles in membrane lipid homeostasis. However, there are no reports to date that plant PAH proteins are associated with LDs, although disruption of the *pah* gene did result in the proliferation of ER membranes, as also seen in yeast (Eastmond et al. 2010).

The involvement of LDs in stress responses has been illustrated by reports that an LD-associated AtCPK1 calcium-dependent protein kinase mediates pathogen resistance in *Arabidopsis* in various tissues, including roots (Coca and Segundo 2010). Interestingly, AtCPK1 regulates several Toll-like interleukin receptors, some of which can trigger immune responses in plants (Ausubel 2005). As noted in "Mammals", bacterial infections in mammals can trigger cytosolic LD formation via Toll-like receptors (Pacheco et al. 2002; Mattos et al. 2010, 2011), which might imply a similar LD-mediated role in pathogen responses in plants and animals. As AtCPK1 can also co-localize with peroxisomes (Dammann et al. 2003), it may play a role in peroxisomal fatty acid ß-oxidation as well as in the release of acyl lipid-derived mediators involved in signalling processes associated with pathogen responses in an analogous manner to those observed in animals (Liang et al. 2003; Shah 2005; Shah and Chaturvedi 2008; Coca and Segundo 2010). Calcium-dependent protein kinases have also been reported on LDs in developing and germinating seeds of sandalwood, *Santalum album* (Anil et al. 2000, 2003), and may be more widespread components of cytosolic LDs in plants.

Mobilization of storage LDs in seeds after germination is a highly regulated process that must occur rapidly in order to supply sufficient energy and acyl chains to developing seedlings. As part of this process, the major LD proteins, oleosin and caleosin, undergo ubiquitination (Hsiao and Tzen 2011), which tags them for proteolytic degradation and thereby enables lipolytic enzymes such as TAG- and PL-specific lipases to access the TAG-rich core of the LDs (Vandana and Bhatla 2006; Quettier and Eastmond 2009; Rudolph et al. 2011). Analysis of the proteome of LDs from maize embryos undergoing post-germinative mobilization revealed two proteins similar to known membrane transport components from animals, namely karyopherin-beta-3 (Kap) and a stress-induced membrane pore protein (Tnani et al. 2011). Kap proteins transport molecules through pores of the nuclear envelope (Mosammaparast and Pemberton 2004), and Kap3 has been shown to be associated with LDs in animals (Cermelli et al. 2006). Human Kap3 interacts with a yeast apolipoproteinA-I (apoA-I), a secretion protein with a primary function in cholesterol transport (Chung et al. 2008). Seed LDs and oleosins are currently being used for various biotechnological applications including the production of recombinant human insulin in plants as well as in cosmetic formulations such as topical creams and lotions (Markley et al. 2006; Nykiforuk et al. 2006; Bhatla et al. 2010; Bonsegna et al. 2011).

Roots and meristems

There are several reports of cytosolic LDs in different cell types in roots (Murphy 2001). A common location is in young roots emerging from seeds shortly after germination, where LDs may be involved in the extensive lipid trafficking required to support the rapid expansion of this tissue. This is substantiated by reports that significant amounts of oleosins and LD-binding caleosin isoforms can be detected in the root tips of 2- to 3-day-old seedlings of rapeseed and *Arabidopsis* (Naested et al. 2000; Hernández-Pinzón et al. 2001). However, LDs may also play other roles in roots. For example, during their initial period of differentiation, root cap cells act as statocytes (gravity sensors). In root cap statocytes of cress, LDs appear to determine a preferential distribution of ER at the distal cell pole and may be one component of the positive orthogravitropic growth of roots (Hensel 1986). Caleosins are also found in young root tips where they may be located either on LDs or on the ER membrane (Hernández-Pinzón et al. 2001; Murphy et al. 2000; 2001). Cells from the shoot apical meristem in birch reportedly contain organelles similar to LDs, and the rearrangement of these structures may be involved in the breakage of bud cell dormancy (Rinne et al. 2002).

Cytosolic LDs may play important roles in meristematic development in plants, as discussed by van der Schoot and Rinne (2011). For example, the dormancy-release mechanism involves the production of numerous LDs, which appear in the cytosol of virtually all meristematic apical cells, but particularly in the RM/RZ (rib meristem/rib zone) and shoot apical meristem regions (Rinne et al. 2002). In the meristematic apex, LDs remain intact throughout the winter and assume peripheral positions where they associ-

ate with the plasma membrane and plasmodesmata (Sargent and Osborne 1980; Vigil et al. 1985; Pihakaski et al. 1987; Rinne et al. 2002), as also occurs in dehydrating seeds (Whitfield 1992; Cordova-Tellez and Burris 2002). This coincides with a restoration of the functionality of plasmodesmata, and it is suggested that LDs may play a direct role in this process (Rinne et al. 2002). These meristem LDs are TAG-enriched and bounded by specific proteins including oleosins, putative lipases and a putative 1,3-ß-D-glucanase. Abundant LDs are also found in several meristematic tissues in pine trees where they appear to function mainly as a winter energy reserve (Jordy 2004). This kind of lipid storage in overwintering tissues has also been found in other gymnosperms such as Douglas fir (Krasowski and Owens 1990) as well as in some deciduous tree species (Catesson 1964; Cragg and Willison 1980).

Cytosolic LDs are highly upregulated as part of the response of many plants to short day lengths. This may be an early downstream effect of photoperiod signalling at the ER where the ethylene receptor required for the timing of dormancy is located (Ruonala et al. 2006; Grefen et al. 2008) and where the LDs are formed. It has also been suggested that LDs may be involved in dormancy processes in root nodules (Gurusamy et al. 2000). The linkage between ethylene and LD induction is supported by the co-expression of genes for ethylene biosynthesis with an LD marker gene in aspen (Rinne et al. 2008). These and other data suggest that the ER and ER-derived LDs might play an important role in the processes that lead up to abscisic acid (ABA)-regulated dormancy processes. As well as inducing oleosin accumulation, ABA also regulates other LD-attached proteins such as some members of the ß-1, 3-glucanase family (Leubner-Metzger and Meins 2000). These finding are consistent with earlier reports that similar LDs are produced during dormancy induction and function primarily as storage organelles that are stimulated to become mobilized by chilling during dormancy release and the subsequent resumption of growth (Rinne et al. 1998; Farrar and Evert 1997; Riding and Little 1984).

Floral tissues

The major lipid-accumulating organs of flowers are the anthers, which are responsible for the development and release of pollen grains. As with seeds, pollen grains are propagules that lead a brief independent existence before germinating on a compatible floral stigma. In plants that produce entomophilous (insect-borne) pollen grains, the tapetal cells of the anther accumulate large amounts of unusual cytosolic LDs (sometimes called tapetosomes). These 1- to 5-μm diameter LDs are composite structures made up of numerous small TAG-rich droplets interspersed with membranous vesicles and tubules (Hsieh and Huang 2005). Associated with these composite LD structures is a class of protein containing a domain with a striking similarity to oleosins that have been termed oleo-pollenins (Murphy 2005, 2006). These proteins are synthesized in tapetal cells and probably bind to tapetal LDs via their oleosin-like domains. However, when tapetal cells undergo apoptosis as part of pollen maturation, the oleosin domains are removed to leave a mature protein, pollenin, which is transferred to the outer wall of the pollen grains (Murphy and Ross 1998).

Pollenins are a diverse class of proteins made up of repeating motifs, often glycine-rich, that resemble structural proteins rather than enzymes. In *Arabidopsis*, pollenins are required for the rapid hydration of pollen grains that is needed for the successful fertilization of female flowers (Mayfield and Preuss 2000). The exact role(s) of pollenins have yet to be determined. They may be involved in pollen rehydration, possibly by facilitating the creation of water channels through the otherwise relatively impermeable lipidic extracellular pollen coat. However, there are also reports of the activation of a pollenin promoter following nematode infection (Karimi et al. 2002). The pollenin genes in some Brassicaceae are reportedly some of the most rapidly evolving genes yet identified (Schein et al. 2004). In addition to their extracellular LD-derived lipids and proteins, pollen grains in many plant species accumulate cytosolic LDs that are bounded by a group of pollen-specific oleosins that are very similar to those expressed in seeds (Kim et al. 2002). These LDs are rapidly mobilized after pollen germination on the female stigma, and the acyl groups contribute to the formation of the long pollen tube that enables haploid pollen nuclei to travel to the ovary and fertilize the female egg and polar nuclei (Murphy 2011).

Plastoglobules

Plants and algae contain an additional organelle that is not present in animals and fungi, namely the plastid. Plastids are the major sites of acyl lipid biosynthesis and the location of the most abundant plant membrane system, the photosynthetic thylakoids. Plastids also contain variable numbers of LDs that are conventionally termed plastoglobules (Bréhélin et al. 2007). As noted above for algae, the plastoglobules of land plants can assume a variety of shapes, including rods and fibres, but are most commonly spherical. They may contain a variety of neutral lipids including TAGs, sterol esters and lipophilic pigments. The colours of most flower petals and other plant tissues are often determined by the pigments contained in their plastoglobules. The major lipid-binding proteins in plastoglobules, the plastoglobulins, belong to a large group of homologous proteins found throughout oxygenic photosynthetic organisms from cyanobacteria to higher plants. This

indicates that their origins may go back to the endosymbiotic bacterial precursors of plastids well over one billion years ago (Kaneko et al. 1996; Hernández-Pinzón et al. 1999; Katz et al. 1995; Pozueta-Romero et al. 1997; Vishnevetsky et al. 1996; Kim et al. 2001).

In addition to forming the major protein component of TAG/pigment-rich fibrils and globules in coloured chromoplasts, plastoglobulins are present in other plastid types such as elaioplasts and chloroplasts (Hernández-Pinzón et al. 1999; Ting et al. 1998; Pozueta-Romero et al. 1997). The plastoglobulins of elaioplasts are located on globular LDs that resemble those of chromoplasts, except that their lipid components are mainly sterol esters and fatty aldehydes (Hernández-Pinzón et al. 1999). In contrast, the plastoglobulins of chloroplasts are associated both with plastoglobules and thylakoid membranes (Pozueta-Romero et al. 1997; Pruvot et al. 1996a, b; Kessler et al. 1999). Plastids from *Brassica rapa* may contain up to three distinct plastoglobulin isoforms, each of which is associated with globules containing a different mixture of neutral lipids (Kim et al. 2001). Plastoglobulins have numerous functions in addition to their structural role of providing a stabilizing surface structure for plastoglobules (Deruere et al. 1994). For example, plastoglobulin gene expression is induced in response to environmental factors such as drought stress, wounding or application of exogenous ABA (Chen et al. 1998; Pruvot et al. 1996a).

Plastoglobulins probably have roles in the formation/disassembly/turnover of plastid membrane complexes (Chen et al. 1998) and in protection against stress-induced uncoupling (Simkin et al. 2007). A plastoglobulin homolog from potato is associated with photosystem II, which is one of the major multi-subunit pigment–protein complexes of thylakoid membranes (Monte et al. 1999). Antisense-mediated reduction of plastoglobulin accumulation in transgenic potato plants led to reduced photosynthetic efficiency and stunted growth, which demonstrates their important roles in plastid membranes and globules. It is likely that there are several classes of plastoglobulins in plants with varying locations in LDs and thylakoid membranes and with varying functions ranging from purely structural to more dynamic roles in protein trafficking and stress responses. The permanent structural coupling between plastoglobules and thylakoid membranes has been demonstrated by high-pressure freezing/freeze substitution methods combined with electron tomography (Austin et al. 2006). This study suggests that the neutral lipids in plastoglobule cores, including carotenoids, plastoquinone and tocopherols, are in a dynamic equilibrium with those located within thylakoid membranes.

As well as plastoglobulins, plastoglobules contain the enzyme tocopherol cyclase (VTE1), which extends across the surface monolayer into the interior of the globules. This enzyme catalyzes the penultimate step of tocopherol synthesis (Kanwischer et al. 2005). It has been shown that tocopherol cyclase activity is increased during oxidative stress, protecting thylakoid membranes and photosynthetic proteins from damage caused by reactive oxygen species (Porfirova et al. 2002; Kanwischer et al. 2005; Vidi et al. 2006). Substantial pools of some of the major lipophilic components of the photosynthetic pigment–protein complexes, such as phylloquinone, are located in plastoglobules (Lohmann et al. 2006). This implies that plastoglobules act as reservoirs to enable a rapid response to environmental conditions by either increasing or decreasing the amounts of such oxidation-prone compounds that in their active state are located adjacent to the vulnerable photosystem proteins.

The plastoglobule proteome also contains several other enzymes involved in lipid metabolism, including allene oxide synthase and a neoxanthin cleavage enzyme (NCED4/CDD4), plus several putative lipases, methyltransferases, steroid isomerases and four putative ABC1 kinases (Vidi et al. 2006; Ytterberg et al. 2006). During senescence, plastoglobules play a final role in the life cycle of the plastids of leaves (chloroplasts) by acting as temporary stores for thylakoid membrane lipids as these are broken down for eventual recycling back to the parent plant before leaf dehiscence. A similar chain of events occurs following the exposure of leaves to ozone, which is a frequent constituent of photochemical pollutants. During these processes, and under other stress conditions, plastoglobulin genes are upregulated via ABA-related hormonal signalling pathways (Pruvot et al. 1996a, b; Chen et al. 1998; Gillet et al. 1998; Manac'h and Kuntz 1999; Kim et al. 2001; Langenkamper et al. 2001; Laizet et al. 2004; Yang et al. 2006). In summary, as with other classes of LDs, plastoglobules have recently emerged as dynamic metabolic compartments that play key roles in a wide range of physiological processes in photosynthetic organisms. Finally, plastoglobules are being investigated as possible targeting sites for the more efficient expression of recombinant proteins (Vidi et al. 2007)

Caleosins

The caleosins are a group of calcium-binding proteins that are probably ubiquitous in multicellular plants, green algae and the true fungi (Naested et al. 2000; Murphy 2005; Partridge and Murphy 2009). Caleosin proteins are characterized by a single calcium-binding EF-hand motif, a putative membrane bilayer spanning domain, plus several potential phosphorylation and haem-binding sites. Structural studies with recombinant seed-specific caleosins indicate that the native proteins are able to bind calcium (Chen et al. 1999; Takahashi et al. 2000), phosphate (Purkrtova et al. 2007) and heme (Hanano et al. 2006). Caleosins appear to be

highly flexible proteins that can dramatically alter their secondary structures in response to the polarity of the medium in which they are embedded (Purkrtova et al. 2007). Heterologous expression of plant LD-binding caleosin isoform in yeast led to the increased accumulation of cytosolic LDs (Froissard et al. 2009), indicating that these proteins might play a generic role in the stabilization of LDs and may also impede their turnover.

Caleosins are frequently described in the literature as LD-associated proteins that occur in storage tissues, such as developing or germinated seeds or caryopses (Liu et al. 2005; Murphy 2005; Toorop et al. 2005) and in somatic embryos (Che et al. 2006). Several caleosin isoforms are also found in the LD proteome (Frandsen et al. 2001; Katavic et al. 2006). However, more recent studies have revealed that although some caleosin isoforms can bind LDs, other isoforms are bilayer-associated enzymes that may be involved in stress responses. Although caleosins have a similar LD-binding proline-rich domain to oleosins, unlike the latter, they appear capable of binding to bilayer membranes as well as to LDs in a similar manner to many animal LD-binding proteins such as the PAT/Perilipin family. In *Arabidopsis*, even the LD-bound isoform, Clo-1, has a calcium-dependent heme oxygenase activity that is regulated by one or two conserved ferric-binding histidine residues (Hanano et al. 2006). This kind of peroxygenase activity may be involved in the formation of epoxy hydroxy alcohols from fatty acid hydroperoxides. These and other oxylipin metabolites play prominent roles in plant responses to a range of biotic and abiotic stresses, from drought tolerance to fungal infection. Similar oxylipins are involved in fungal spore development, and those produced in plants probably serve as antifungal compounds to deter the growth of competing fungal species (Tsitsigiannis and Keller 2007). Another *Arabidopsis* caleosin isoform, Clo-3, is involved in stomatal control, transpiration, drought tolerance and fungal resistance (Aubert et al. 2010), whilst Clo-4 can act as a negative regulator of ABA responses (Kim et al. 2011).

Protists and fungi

This section covers eukaryotes except for the animals and plants. It is mainly concerned with simple protists and with the large group of fungi that range from the unicellular yeasts to relatively complex and large multicellular organisms such as Basidiomycetes. Most or all of these organisms are able to accumulate cytosolic LDs, and one of the first indications that intracellular TAG pools might be actively involved in phospholipid metabolism came from studies of the ciliated protozoan, *Tetrahymena pyriformis*, as long ago as 1976 (Borowitz and Blum 1976). In this study, it was found that *T. pyriformis* contained a very labile TAG pool, separate from other endogenous TAG pools, and that both the glycerol backbone and the acyl groups of this labile pool served as precursors for membrane phospholipid biosynthesis. These early results already suggested the sort of intimate relationship between TAG and phospholipid metabolism that has subsequently been observed in many other organisms. For an up-to-date account of the metabolic regulation of TAG formation in heterotrophic microbes, see the review by Kosa and Ragauskas (2011).

Many protists and fungi act as parasites or pathogens, and the ability to accumulate cytosolic LDs is often a key part of their success as infectious agents. Moreover, several of these organisms are able to stimulate the formation of LDs in host cells that are then mobilized as energy sources by the parasite or pathogen. Some of the best studied organisms are the apicomplexan parasites of the *Plasmodium* and *Toxoplasma* genera (Vielemeyer et al. 2004; Coppens and Vielemeyer 2005; Coppens 2005). In the case of the malarial parasite, *Plasmodium falciparum*, an essential factor for the proliferation of the parasite within infected human erythrocytes is its ability to induce the accumulation and subsequent mobilization of large amounts of TAG (Palacpac et al. 2004). Whilst some of the derived acyl groups are transferred to the parasite and accumulate in its cytosol as TAGs, many are released into the infected erythrocyte as the parasite reaches the schizont stage. This sudden release of fatty acids may cause the membrane lysis that leads to cell rupture and the release of merozites (Palacpac et al. 2004). Therefore, in this case, host LDs appear to function not just as a nutrient source for the parasite but also as a cellulolytic mechanism to enable *P. falciparum* cells to escape from host cells and enter the next phase of their life cycle.

Other parasitic protozoans, such as *Trypanosoma brucei* (the cause of human African trypanosomiasis), accumulate large numbers of LDs during infection of their host. Unusually, a novel protein kinase (LDK) in *T. brucei* has been found to be an LD-binding protein, and its RNAi-mediated knockdown resulted in a greatly reduced abundance of LDs (Flaspohler et al. 2010). Whilst previous studies had shown some association of mitogen-activated protein kinases with leukocyte LDs (Yu et al. 1998), this was the first report of a kinase that could strongly bind to LDs and had an important role in their function. The exact role of LDK in regulating LD function remains to be determined, but it seems likely that it will involve the phosphorylation of proteins involved in LD formation/turnover.

There have been relatively few recent studies on the occurrence and function of cytosolic LDs in free-living protists, particularly in comparison with the much better characterized prokaryotes, animals and plants. However, it now appears likely that protists share a common mode

of LD regulation with all other Unikonts. The Unikonts are a supergroup that includes Amoebozoa (e.g. slime moulds), Metazoa (multicellular animals), and Fungi (including secondarily reduced Microsporidia; Keeling et al. 2000; Keeling and Fast 2002; Lee et al. 2008; Koonin 2010). The emerging evidence of a common method of LD regulation comes mainly from comparative genomics, which has revealed the occurrence of *PAT/perilipin*-like genes in members of each of these very diverse groups of Unikont organisms. In much of the literature after 2000, the term 'PAT proteins' was commonly used, but this has now been superseded by Perilipin, as described in the box below.

Perilipins (PAT proteins) – the major LD proteins in Unikonts

In this article, the recently proposed nomenclature for PAT proteins (Kimmel et al. 2010) will be adopted. According to this system, all PAT proteins are now called perilipin, or Plin, followed by a number, as follows:

- Plin 1 corresponds to Perilipin/LSD1
- Plin 2 is ADRP/adipophilin/fatvg/LSD2
- Plin 3 is TIP 47/PP17
- Plin 4 is S3-12
- Plin 5 is MLDP/OXPAT/LDSP5

The PAT/Perilipin group of proteins was first described in mammals and is known to be involved in many aspects of LD organization and function (Greenberg et al. 1991; Brasaemle et al. 1997). More recently, the occurrence of PAT-like genes in all Unikonts (although some of these genes have yet to be correctly annotated) has provided evidence that a PAT protein-based mechanism for LD function originally evolved in the common ancestor of extant Unikonts, from slime molds to humans (Miura et al. 2002; Bickel et al. 2009; Kimmel et al. 2010). The genomes of all non-mammals, such as insects, analyzed to date only contain *Plin1* and *Plin2* orthlogs, which implies that *Plin3-5* may be unique to mammals. In some mammalian genomes, the *Plin 3, 4,* and *5* genes are adjacent and probably arose due to duplication of a primordial *Plin3* gene. Perilipin 5 is the most recently established family member and its function has yet to be determined. In mice *Plin5* mRNA expression is restricted to oxidative tissues, such as heart, slow-twitch fibers of skeletal muscle, brown adipose tissue, and liver and the gene is strongly induced by fasting (Dalen et al. 2007; Wolins et al. 2006; Wang and Sztalryd, 2011).

Fungi

Cytosolic LDs can be found in the majority of fungal cells where their functions may vary according to the species, developmental stage and/or environmental conditions. For example, cytosolic LD formation commonly occurs during vegetative growth in saprophytic fungi, but LD numbers also increase markedly during the formation of resting and reproductive structures (Murphy 2005). The pathogenic fungus, *Plasmodiophora brassicae*, inserts itself into the cytosol of its *Brassica* plant host, whereupon it rapidly accumulates LDs in its own cytosol (Lösel and Sancholle 1996); these lipids are temporary carbon stores synthesized from precursors extracted from the host plant. An unusual function of fungal LDs is found in sporangiophores of the unicellular fungus, *Phycomyces blakesleeanus*, which contain aggregates of several dozen 1- to 2-μm diameter LDs, possibly tethered by microfilaments, which may play a role in gravity sensing (Schimek et al. 1999). Fungal LDs appear to arise from the ER in a similar manner to other eukaryotes (Schneider and Seaman 1977).

Some fungi, such as the ascomycete, *Metarhizium anisopliae*, contain a single PAT/Perilipin gene corresponding to *Plin1*, which encodes an LD-associated protein with a role in TAG storage (Wang and St Leger 2007). More recently, the strong coupling of LD formation and function with the ER

has been investigated in more detail, and it has been shown that in cells that lack LDs, proteins normally associated with LDs are instead evenly distributed in the ER membrane (Jacquier et al. 2011). These studies show that transcriptional induction of the diacylglycerol (DAG) acyltransferase, Lro1, is sufficient to drive LD formation on ER membranes where nascent LDs become decorated by marker proteins. When LD formation is induced by the expression of a second DAG acyltransferase, Dga1, this enzyme moves from the ER membrane to LDs as they bud off and move into the cytosol. Photobleaching studies indicate that the movement of proteins from the ER to LDs is independent of temperature and energy, and thus not mediated by classical vesicular transport routes. In some cases, LD-localized proteins can relocate back to the ER, indicating that some continuity between the two organelles is maintained, even if only transiently, in a way that allows the two-way partitioning of proteins between the two compartments.

A novel aspect of LDs in some mycorrhizal fungi is their role as long-range transportable food reserves. Whilst most fungi translocate simple carbohydrates, in some species, such as *Glomus intraradices* and *Glomus margarita*, the majority of carbon is translocated as LDs that can comprise as much as 16% of the hyphal volume. Time-lapse micrographs show translocated LDs moving along specific tracks within the hyphal cells at speeds of up to 11 μm s^{-1} (Bago et al. 2002). It is calculated that for each of the principal fungal hyphae, as much as 1.3 μg h^{-1} TAG is transported in this way. The movement of the LDs along specific tracks, independently of cytoplasmic streaming, suggests an organized transport system, possibly via cytoskeletal elements, as found for some LDs in animal cells (Murphy 2001).

Many pathogenic fungi use specialized structures, termed appressoria, to break through the tough surfaces of their hosts. Lipid droplets appear to be crucial for appressorium function and especially in the production of the high turgor pressure that is required for virulence (Thines et al. 2000; Wang and St Leger 2007). In the rice blast fungus *Magnaporthe grisea* (Thines et al. 2000) and the insect pathogen *M. anisopliae* (Wang and St Leger 2007), lipid droplets originate in fungal spores and redistribute to the incipient appressorium. Whilst the underlying mechanism is unknown, several kinases are implicated (Thines et al. 2000). LDs also play roles in colonization and sexual development in other fungi, including the wheat pathogen, *Fusarium graminearum* (Guenther et al. 2009), and *Aspergillus nidulans* (Tsitsigiannis et al. 2004), which is a soil-dwelling fungus and an opportunistic human pathogen.

A. nidulans can produce either sexual or asexual spores according to environmentally determined hormone-like signals generated from oxylipins. A putative fatty acid dioxygenase involved in the biosynthesis of oxylipins from linoleic acid, PpoA, has been shown to be an LD protein (Tsitsigiannis et al. 2004). The deletion of this protein resulted in reduced oxylipin levels and a sixfold decrease in the ratio of asexual to sexual spores. This suggests that the formation of oxylipins may occur at the surface of cytosolic LDs, which are abundant in spore-producing fruiting bodies of most fungi. LDs also play a role in virulence in the human pathogen, *Candida parapsilosis*, as shown when the gene encoding the LD-associated Fat storage-Inducing Transmembrane (FIT2) protein was disrupted (Nguyen et al. 2011). Mutated cells showed greatly reduced TAG and LD accumulation, lower growth rates in nutrient-rich media and a much attenuated pathogenicity in murine infection models. In *C. parapsilosis*, the accumulation of LDs protects cells against glucotoxicity- or lipotoxicity-induced by exposure to elevated levels of glucose or fatty acids in growth media in a process that also involves acyl desaturases (Nguyen and Nosanchuk 2011).

The yeast model, *Saccharomyces cerevisiae*

Due to its ease of cultivation, the brewers' yeast, *Saccharomyces cerevisiae*, is one of the best characterized model eukaryotes. Recent studies have demonstrated that this relatively simple organism shares many features of LD organization and function found in much more complex animals such as insects and mammals. Some yeast LDs contain only either TAGs or sterol esters (SEs; Ducharme and Bickel 2008; Horn et al. 2011), whilst others have a mixed composition (Czabany et al. 2008). Evidence from physical probes of LDs isolated from mutants unable to synthesize TAGs or sterol esters SEs suggests that these two lipid classes are partially segregated within the LD core, with thin shells of SEs forming concentric hollow spheres around an inner core composed principally of TAGs. Further evidence that LDs may not always contain a homogeneous core of mixed neutral lipids undergoing isotropic motion has come from the observation that they sometimes contain electron-dense material within their cores (Czabany et al. 2008). These so-called gnarls consist of tangles of elongated and curled tubules of diameter about 10–30 nm, and it was speculated that they might be lipid metabolites, such as free fatty acids, that had demixed or phase-separated from the isotropic TAG/SE-rich core. Similar observations relating to the possibly non-homogeneous structure of some LD cores have also been reported in mammalian systems (Robenek et al. 2004, 2005a, b; see also "Mammals").

Analyses of the proteomes of yeast LDs and peroxisomes, as verified by microscopic immunodetection, have revealed the presence of numerous lipid metabolism and ER-related proteins (Athenstaedt et al. 1999; Binns et al. 2006). One notable absence is of any PAT/Perilipin

homologs, in contrast to their presence in ascomycete fungi such as *M. anisopliae*. Among the most abundant LD proteins in yeast are three enzymes involved in the synthesis of its major neutral lipid component, ergosterol, namely sterol Δ24-methyltransferase, squalene epoxidase and lanosterol synthetase, as well as three enzymes of long-chain fatty acid activation. The yeast LD proteome also contains several peroxisomal and mitochondrial proteins as well as membrane-trafficking proteins, nuclear proteins, chaperones, enzymes and plasma membrane-associated proteins. The presence of peroxisomal proteins is consistent with the observed close physical association between LDs and peroxisomes in yeast, where the latter have been found to extend tubular projections called 'pexopodia' into adjacent LDs (Binns et al. 2006). The involvement of yeast LDs with ER function has been underscored by the reported association with the LD phospholipid monolayer of three proteins that are functionally linked to ER-associated degradation (ERAD) proteins, namely UBXD8 (Zehmer et al. 2009a), AUP1 and UBE2G2 (Spandl et al. 2011). However, another recent report suggests that LD formation is not essential for ERAD in yeast (Olzmann and Kopito 2011). Although the yeast genome does not have any orthologs of the abundant fugal and plant LD-binding protein, caleosin, the expression of a plant caleosin isoform in yeast cells led to the much increased accumulation of cytosolic LDs (Froissard et al. 2009). Therefore, the mere presence of this foreign protein caused yeast cells to overaccumulate LDs. The absence of *caleosin* genes in the yeasts, but their presence in the filamentous fungi and plants, may be due to the selective loss of these genes from yeasts at some point during their evolution.

Yeast cells are able to take up free fatty acids, which are activated to acyl-CoAs and either catabolized via ß-oxidation or converted to complex lipids. In the yeast, *Yarrowia lipolytica*, the synthesis of TAG and the formation of LDs are regulated in part by acyl-CoA oxidases (Mlíčková et al. 2004a, b), although it is not known whether this applies to other species as well. If LD formation is blocked in yeasts, as in the *S. cerevisiae* quadruple mutant strain, *dga1:lro1:are1:are2*, exogenous fatty acids become toxic when they are taken up by cells (Lockshon et al. 2007; Petschnigg et al. 2009). This indicates that one of the constitutive functions of LDs is to maintain intracellular lipid homeostasis, and especially to minimize the risk of fatty acid toxicity by serving as a reservoir for the sequestration of excess acyl groups. However, unlike many animal cell lines, yeast cells are able to adapt to the presence of high levels of exogenous fatty acids even if LD formation is blocked (Connerth et al. 2010). In this case, it was observed that yeast was instead able to hyper-accumulate membrane lipids, mainly by proliferation of the ER network. Some exogenous fatty acids also altered the composition of LDs in yeast; hence, oleate feeding led to a 16:1 ratio of TAG/SE, whilst this ratio was near the wild-type value of 1:1 in the presence of palmitate (Connerth et al. 2010). This was probably due to the need to balance excessive membrane fluidity caused by high levels of oleate by suppressing SE formation so that relatively rigid free sterols could be incorporated into membranes. In this case, the LDs are playing an indirect role in the maintenance of optimal membrane fluidity in the face of environmental challenges caused by different types of exogenous lipid substrate.

Further parallels between yeast and mammals are shown by the presence in yeast of homologs of LD-associated proteins involved in lipodystrophy in humans, such as seipin (Fei et al. 2008, 2011a) and lipin (Han et al. 2006; Adeyo et al. 2011). As discussed in "Mammals", mutations in these genes in humans can result in defective adipogenesis with often severe clinical outcomes (Agarwal and Garg 2006). In yeast, a functional homolog of human seipin, Fld1p, regulates the LD size (Fei et al. 2008), which is consistent with its proposed role in humans in the assembly and maintenance of LDs (Binns et al. 2010). The yeast ortholog of lipin, Pah1p, is a phosphatidate phosphatase responsible for DAG formation. Whilst DAG produced by Pah1p is one source of TAG for LD formation, there are alternative pathways for TAG production in yeast (Murphy 2001). Deletion of Pah1p in yeast reduced but did not abolish TAG formation, and the cells responded by synthesizing more SE in order to maintain normal amounts of LDs and neutral lipids (Adeyo et al. 2011). In some cases, yeast Pah1 mutants also hyper-accumulated membranes of their ER-nuclear envelope networks (Han et al. 2006, 2007a; Santos-Rosa et al. 2005). However, when both Pah1p and the sterol acyltransferases Are1p and Are2p were deleted, no LDs were formed even though some TAG was still available. It is proposed that Pah1p-derived DAG plays a key role together with Are1p and Are2p in the formation of LDs on the ER membrane, as depicted in Fig. 3 (Adeyo et al. 2011).

The role of the seipin homolog in yeast, Fld1p, was confirmed by a recent screen of mutants that accumulate 'supersized' LDs, which are generally about 50-fold larger in volume than wild-type LDs (Fei et al. 2011b). In this study, ten mutant lines were found with 'supersized' LDs and the causal genetic lesions identified. One of the mutated genes encoded Fld1p, but another five genes (*CDS1*, *INO2*, *INO4*, *CHO2* and *OPI3*) encoded known enzymes of phospholipid metabolism, whilst two genes (*CKB1* and *CKB2*) encoded subunits of the casein kinase 2. A common feature of these mutants was their increased levels of phosphatidic acid that were shown to facilitate the coalescence of LDs into much larger structures that were more difficult for lipases to access. Fld1p is not an enzyme

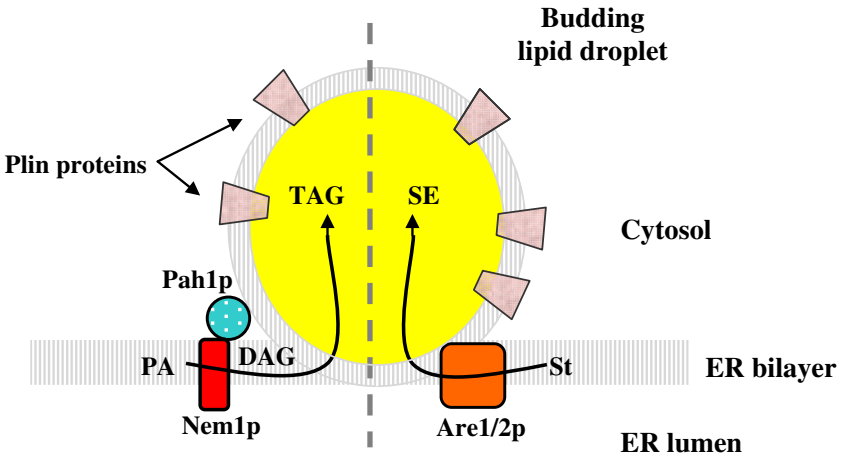

Fig. 3 Model for lipid droplet formation in yeast. In this theoretical model, sterol ester-enriched LDs are formed via the action of the enzyme, Are1/2p, which esterifies ER-located sterol precursors. Alternatively, TAG-enriched LDs are formed from PA, via DAG, by the acyltransferases, Pah1p and Nem1p. In both cases, the curvature of the outer bilayer leaflet of the ER that is required for directional budding of the nascent LD towards the cytosol may be facilitated by the localized accumulation of non-bilayer-forming lipid intermediates such as PA, DAG, and SE as well as the action of the transbilayer proteins in blocking lateral diffusion of lipid intermediates away from the site of LD formation. Most yeast LDs contain either SE or TAG cores (yellow) rather than a mixed composition. However, mixtures of SE and TAG are frequently found in LDs of higher animals and plants. Figure adapted from Adeyo et al. (2011)

of lipid metabolism, and the mechanism by which it regulates LD size has yet to be determined, although its homolog in *Drosophila* may have a role in phosphatidic acid metabolism (Tian et al. 2011).

The overall process of LD turnover in yeast has clear similarities with that in mammals. This is seen by the presence of very similar enzymes of TAG breakdown and the conservation of mechanisms for lipolysis between yeast and mammals (Kurat et al. 2006, 2009). These observations are important because the hyper-accumulation of LDs and the proliferation of adipose tissue are key aspects involved in human obesity, and yeast could be a valuable model system for studying the cellular basis of this dysfunction. The process of LD lipolysis in yeast has been linked to cell cycle progression via a common Cdk1/cdc28 activation mechanism (Kurat et al. 2009), whilst a novel lipid hydrolase with esterase and TAG lipase activities has been shown to be involved in the overall cellular lipid homeostasis (Debelyy et al. 2011). Other studies have implicated LD dynamics and their interactions with peroxisomes and ER in the ageing process in yeast (Goldberg et al. 2009) in an analogous manner to that proposed for multicellular organisms, including mammals (Haemmerle et al. 2006).

The proposal from these and other studies is that LDs in yeast cells function as a hub in a regulatory network that modulates TAG and SE formation in the ER and fatty acid oxidation in peroxisomes. When yeast cells are exposed to a calorie-rich diet, it is proposed that the resultant accumulation of ethanol represses the peroxisomal oxidation of free fatty acids that originate from TAGs synthesized in the ER and deposited within LDs. The free fatty acids will then build up inside LDs to form the 'gnarl' structures discussed above. The presence of gnarls then initiates several negative feedback loops, resulting in the hyper-accumulation of TAGs in LDs and of DAGs and free fatty acids in the ER. As discussed by Goldberg et al. (2009), this can potentially lead to a cascade of metabolic and physiological events as follows: (1) loss of peroxisome function triggering necrosis; (2) lipoapoptosis induced by free fatty acids and DAG (a caspase- and mitochondria-independent form of programmed cell death); and (3) the triggering by DAG of a protein kinase C-dependent signal transduction network affecting multiple stress response- and longevity-related processes. Therefore, the ingestion of a calorie-rich diet by yeast cells may considerably shorten their life span due to LD-associated metabolic changes in a manner that has many analogies with similar processes in animals, including humans, where caloric restriction is correlated with increased longevity (Bordone and Guarente 2005; Feng et al. 2007; Puigserver and Kahn 2008).

As with several other efficient producers of LDs, some yeast species are being used for biotechnological purposes such as the breakdown and utilization of hydrophobic substrates (Kosa and Ragauskas 2011). Some species can accumulate lipids to as much as 70% of their biomass. One of the most promising of these lipogenic systems is *Y. lipolytica* whose potential role as a model for bio-oil production has recently been reviewed (Beopoulos et al. 2009). Other yeasts being used or investigated for commercial lipid production from organic wastes or simple low-cost substrates include *Candida* spp, *Cryptococcus curvatus*, *Lipomyces starkeyi*, *Rhodotorula* spp. and *Trichosporon*

spp., whilst filamentous fungi also in use include *Aspergillus oryzae*, *Mortierella* spp. and *Rhizopus* spp. (Kosa and Ragauskas 2011). The most efficient commercial yeasts, *C. curvatus* and *L. starkeyi*, produce slightly higher lipid yields than the best bacterial systems, which are *Rhodosporidium toruloides* and *R. opacus* PD630 (see "Prokaryotes").

Lower animals

All metazoans are able to accumulate cytosolic LDs containing TAG and/or SE in most or all of their cells. For example, LDs are ubiquitous organelles throughout the body of the nematode worm, *Caenorhabditis elegans*, although they are especially abundant in the fat-storing cells of the gut epithelium (Zhang et al. 2010b). As also described above for yeast, LDs in *C. elegans* play an important role in its longevity (Feng et al. 2007; Wang et al. 2008). Hence, mutants with impaired peroxisome function will hyper-accumulate LDs, leading to developmental abnormalities (Zhang et al. 2010a). As with the fungi, LD function in animals is tightly coupled with cycles of biosynthesis and lipolysis involving the ER and peroxisomes, with PAT/Perilipin proteins playing key roles in these processes. In recent years it has become apparent that not all LDs within a given cell are identical. For example, some LDs in the same cell may carry different proteins (Beller et al. 2010a; Wolins et al. 2006a, b; Beller et al. 2006; Ozeki et al. 2005), whilst some LDs in the same cell can also carry different lipid cargoes (Czabany et al. 2008).

The insect model, *Drosophila melanogaster*

The vast majority of research relating to invertebrate LDs has been done using the model fruit fly, *Drosophila melanogaster*. The importance of LDs in the overall function of *Drosophila* has been demonstrated by data from two RNAi screens that uncovered a large and diverse array of proteins and organelles that participate in LD physiology. It was found that as much as 1.5% of the expressed genome is implicated in LD function, totaling approx. 370 genes (Beller et al. 2008; Guo et al. 2008). The important and increasing contribution of the *Drosophila* model to wider LD research has been reviewed by Kühnlein (2011).

Early-stage *Drosophila* embryos were one of the first systems where it was shown that cytosolic LDs are able to move bidirectionally within cells in association with dynein, a motor protein that can move along microtubules whilst carrying a cargo (Gross et al. 2000). More recently, it has been shown that kinesin-1 also participates as a motor protein to drive LDs along microtubules (Shubeita et al. 2008). During *Drosophila* oogenesis, LDs and most other oocyte contents are first formed in nurse cells and then transferred through cytoplasmic bridges to the oocyte. This process involves actin-based cytoplasmic streaming (Gutzeit 1986) and active transport along microtubules (Gross et al. 2000). As nurse cell components arrive in the oocyte, they are thoroughly mixed via large-scale, microtubule motor-driven cytosolic streaming. Although most oocyte LDs are carried passively by the bulk flow of streaming, a subset moves actively and bidirectionally along microtubules (Gaspar and Szabad 2009). In early embryos, virtually all LDs move bidirectionally along radially organized microtubules. Embryos may be able to compensate for abnormal LD distribution since embryos with defects in various aspects of LD orientation appear to develop normally (Welte et al. 2005). In addition to dynein, *Drosophila* LDs bind the Klarsicht (Klar) protein, which, as its name suggests, was originally identified in photoreceptors where it functions as a nuclear envelope-located motor regulator. However, other isoforms of Klar are targeted to LDs in embryos, ovaries and several somatic tissues where they regulate the bidirectional motion of LDs via both dynein and kinesin-1 (Yu et al. 2011).

The most common neutral lipid in insect LDs is TAG, although SEs may be present in some tissues. As in nematodes, insect LDs appear to be ubiquitous throughout the body, but accumulate to very high levels in specialized storage tissues such as the fat body, as reviewed by Arrese and Soulages (2010). Despite its name, the insect fat body is a complex multifunctional organ involved in the coordination of growth with metamorphosis and reproduction. Its main function is to store and release compounds that play important roles in these processes. For example, in *Aedes aegypti* females, the fat body synthesizes vitellogenin in a transcriptionally mediated process that responds to the ingestion of a blood meal (Park et al. 2006). By far, the most common cells in fat bodies are the highly LD-enriched cells termed adipocytes. However, as they contain numerous small LDs, these insect cells are more similar to mammalian brown adipocytes rather than the far commoner white adipocytes where most mammalian storage lipid is deposited (see "Mammals"). Insects that fly for extended periods, such as during migrations, are able to use LDs directly to power their flight muscles. In such cases, LD TAG is converted into DAG and secreted into the hemolymph from where it is transported to flight muscles via the lipid carrier protein, lipophorin (Arrese and Soulages 2010).

The proteome of *Drosophila* LDs is similar to other animals in containing PAT/Perilipin family proteins, although only Plin1 and Plin2 are present, with Plin3–5 apparently restricted to vertebrates. The important roles of the PAT/Perilipin family proteins in *Drosophila* have been shown by abnormalities in LD formation and function that are found in mutants (Grönke et al. 2003; Teixeira et al. 2003). A role for

Plin1 in LD homeostasis is suggested by the finding that it is downregulated in starvation (Grönke et al. 2005). More recently, it has been shown that the Plin1 homolog in *Drosophila* regulates LD structure and the access of other proteins to the LD surface (Beller et al. 2010a). In particular, some Plin1-deficient mutants had much larger LDs than wild-types, and in some cases, fat body cells were dominated by a single giant LD reminiscent of mammalian white adipocytes. These mutants were also characterized by an obesity phenotype that was clearly related to LD dysfunction and suggests that LD status can determine feeding behaviour, possibly via an adipokine or other signal from fat bodies (Grönke et al. 2007; Beller et al. 2010a). However, a knockout of both Plin1 and Plin2 only resulted in reduced fat body stores and reduced ability to mobilize these upon starvation. These results, and the fact that Plin homologs are absent from yeasts and *C. elegans*, show that Plin proteins are not essential for LD formation or survival of the organism, especially under favourable feeding conditions. However, they do appear to play key roles as potentiators of lipid metabolism and may have particular selective advantages under conditions of limited or varied access to food (Beller et al. 2010a).

Similar to fungi and mammals, *Drosophila* LDs contain homologs of the protein, Seipin, and knockout of the *Seipin* gene results in a much reduced accumulation of LDs in the fat body and their presence in ectopic locations such as salivary glands (Tian et al. 2011). The reduction in LD size and numbers was due to the lower rates of TAG formation rather than increased lipolysis. Genetic evidence suggests that aberrant forms of Seipin may cause a reduction in the activity of TAG biosynthetic enzymes, leading to lower levels of LD production. Another novel LD-associated protein recently found in *Drosophila* is the conserved metalloprotease, invadolysin (Cobbe et al. 2009). Invadolysin is a member of the M8 family of metzincin metalloproteinases (Gomis-Rüth 2003). Homologs of invadolysin are found in many different organisms and probably regulate the function of other proteins by cleaving them to generate active forms. In several protozoan parasites, invadolysin homologs are important in conferring virulence, whilst invadolysin mutants of *Drosophila* die in late larval stages (McHugh et al. 2004). Larvae of mutants in which invadolysin was undetectable had reduced fat bodies and lower TAG levels in a similar manner to that seen in PAT/Perilipin deficient mutants (Grönke et al. 2003; Teixeira et al. 2003).

Several studies of the *Drosophila* LD proteome at different developmental stages have shown the presence of expected components such as the PAT/Perilipin group, lipid metabolism enzymes and intracellular transport proteins, but also less predictable components such as storage proteins and histones (Beller et al. 2006; Cermelli et al. 2006). Further studies have demonstrated that these unexpected proteins have a genuine association with LDs in vivo. It is proposed that proteins such as histones are sequestered on LDs in embryonic cells to provide a readily accessible store that can be used at short notice. It may be necessary to sequester highly regulated proteins such as histones in this way in order to shield them from the efficient surveillance mechanisms that normally detect and destroy surplus, damaged or misplaced proteins (Gunjan and Verreault 2003). Similar roles for LDs as protein storage/recycling sites have been proposed in mammalian systems (Fujimoto and Parton 2010). In other cases, LDs may act as long-distance carriers of large protein cargoes along the cytoskeletal network. This mechanism may be particularly relevant during early embryo development when there is a great deal of directional movement of cellular components (Cermelli et al. 2006). The LD proteome from third-instar fat body cells did not include histones, but several storage proteins were present instead, as well as chaperones, including three heat shock proteins (Beller et al. 2006). In general, the *Drosophila* LD proteome is strikingly similar to that of mammals, which indicates both their evolutionarily conserved functions and the potential utility of *Drosophila* as a model to investigate LD malfunction in serious human pathologies such as metabolic syndrome and lipodystrophy.

An example of the utility of *Drosophila* as a model for studying LD function and regulation comes from studies that have elucidated the role of the coat protein I (COPI) complex as a regulator of lipid homeostasis (Beller et al. 2008). COPI and COPII vesicles are essential components of the trafficking system between the ER and Golgi (Lee et al. 2004). COPI vesicles mediate retrograde cargo transport from the Golgi back to the ER, including escaped ER-resident proteins. The anterograde counterpart, COPII, mediates the transport of proteins and lipids from the ER to the Golgi. If either COPI or COPII complexes are disrupted, the Golgi function is abolished, but only COPI is required for LD mobilization. In the absence of COPI function, LDs accumulate to abnormal levels. COPI components changed the LD protein coat composition, particularly by removing Plin3 and hence promoting lipase binding and the initiation of TAG hydrolysis. Following preliminary experiments in *Drosophila*, studies in mammalian cell lines demonstrated that COPI-mediated regulation of LD turnover is a general mechanism common to all animals (Beller et al. 2008).

Mammals

There have been numerous recent reviews focusing on mammalian LDs (Ducharme and Bickel 2008; Fujimoto et al. 2008; Goodman 2008; Thiele and Spandl 2008; Walther

and Farese 2009; Bickel et al. 2009; Farese and Walther 2009; Guo et al. 2009; Murphy et al. 2009; Ohsaki et al. 2009; Olofsson et al. 2009; Zehmer et al. 2009b; Beller et al. 2010b; Bozza and Viola 2010; Fujimoto and Parton 2010; Kalantari et al. 2010; Greenberg et al. 2011; Hapala et al. 2011), plus several useful pictorial overviews of lipid trafficking in the mammalian cell (Krahmer et al. 2009; van Meer and de Kroon 2011). The roles of cytosolic LDs in the assembly and turnover of the various classes of secreted lipoproteins in mammals have been described elsewhere and will not be discussed further here (Olofsson and Boren 2005; Shelness and Ledford 2005; Ohsaki et al. 2006, 2008, 2009; Olofsson et al. 2009).

The emerging picture is that mammalian cells have the same basic LD system as other animal groups, such as the insects that are described above. However, in mammals, these basic LD mechanisms are supplemented by additional layers of complexity and functional redundancy that have been adaptive during the evolution of this group of relatively large and long-lived eukaryotes. For example, mammalian cells appear to have several distinct global mechanisms for the formation and turnover of LDs that are regulated by different effectors and/or are active in different tissues. Even at the metabolic level, mammals tend to have more forms of key enzymes, such as DAG acyltransferase (for LD formation; Harris et al. 2011) or lipases (for LD turnover; Osuga et al. 2000; Jenkins et al. 2004; Villena et al. 2004; Zimmermann et al. 2004; Quiroga and Lehner 2011). This means that multiple gene knockouts are frequently required to halt such processes. The LD population of individual mammalian cells can be heterogeneous, as demonstrated in isolated 3T3-L1 adipocytes which contained small LDs mainly loaded with Plin3/4, intermediate-sized LDs mainly loaded with Plin2 and large LDs mainly loaded with Plin1 (Wolins et al. 2005).

The composition and structure of the core of mature LDs in mammals has been the subject of some discussion in the literature. It was originally believed that all LDs had a relatively simple structure made up of an isotropic core of neutral lipid surrounded by a phospholipid monolayer and various coat proteins (Murphy and Vance 1999). However, there is increasing evidence that in some mammalian cells, normally soluble or amphipathic proteins (Dvorak et al. 1994; Bozza et al. 1997; Robenek et al. 2005a, b, 2006), and even entire ribosome-bound membranes (Wan et al. 2007), may sometimes partition into supposedly hydrophobic LD cores (Ohsaki et al. 2009). In particular, there have been several reports of the presence of caveolin-1 in LD cores, which is puzzling because caveolins are believed to be transferred to the LD surface either via lateral diffusion from the ER membrane or from the plasma membrane via the endocytic pathway (Fujimoto et al. 2001; Pol et al. 2001; Ostermeyer et al. 2001, 2004; Le Lay et al. 2006). Several lines of ultrastructural evidence from the group of Robenek et al. (2004, 2005a, b, 2011) are consistent with the presence of LD coat proteins, such as Plin1 and Plin2, as well as caveolin-1, within LD cores. Given recent evidence of the possible existence of distinct lipid domains, such as the fatty acid 'gnarls' found in yeast LD cores (Czabany et al. 2008), it is clear that the nature of LD cores in mammals and in other organisms requires further research.

Heterogeneity of mammalian LDs

The morphology and function of mammalian LDs tend to be more variable than those of other organisms. For example, mammals have two broad types of highly LD-enriched storage cells, namely white and brown adipocytes, which have very different LD structures and physiological functions. In mammals, cytosolic LDs are also prominent constituents of steroidogenic cells in the adrenal gland or reproductive organs (for steroid hormone synthesis), mammary gland epithelial cells (for milk fat synthesis), hepatocytes and enterocytes (for lipid metabolism and lipoprotein formation), and leukocytes (for synthesis of eicosenoid mediators; Murphy 2001). All of these roles are in addition to those of the constitutive LDs that are present in all cells and now known to have a dynamic involvement in lipid trafficking in all eukaryotes. More recently, the list of processes involving LDs in mammalian cells has expanded considerably, as has the number of proteins that are bound to or otherwise associated with these organelles (Beller et al. 2010b; Itabe 2010).

For example, multifunctional LDs are required in different cell types to store and release acyl groups that have been tailored to accommodate cell-specific requirements and functions (Wang and Sztalryd 2011). Such functions include rapid fatty acid storage/release from or into blood by white adipocytes (Ahmadian et al. 2010), mitochondrial fatty acid oxidation for thermal regulation by brown adipocytes (Ricquier 2010), fatty acid oxidation for long-term mobility demands by slow-twitch fibres in skeletal muscles (Moro et al. 2008), lipidation of very low-density lipoprotein production and mitochondrial ß-oxidation (Olofsson et al. 2009), milk production in mammary epithelial cells (Keenan and Mather 2006; Chong et al. 2011a, b) and surfactant production in type II alveolar pneumatocytes in the lung (Magra et al. 2006). Mammalian LDs have been implicated in acyl lipid turnover (Smirnova et al. 2006), cholesterol homeostasis (Naslavsky et al. 2007), hepatitis C infection (Miyanari et al. 2007), proteasomal and lysosomal degradation of apolipoprotein B (Ohsaki et al. 2006), and protein phosphorylation and GTP-mediated protein trafficking (Bartz et al. 2007c; Hommel et al. 2010). It is also now clear that brown adipocytes with their small and readily accessible LDs

have roles in energy homeostasis in human adults, in addition to their well-known thermogenic roles in foetuses and neonates (Cypess and Kahn 2010).

As in other animals, the major group of LD-associated proteins in mammals is the PAT/Perilipin family, but the remainder of the mammalian LD proteome tends to be more variable and considerably larger than in non-mammals such as *Drosophila* (Hodges and Wu 2010). In addition, the LD proteome can vary significantly according to tissue, developmental stage and physiological status, and the list of novel LD-associated proteins only found in mammals continues to grow. Similar to other animals, the abundant LD-binding phosphoprotein, Plin1, has a major role in regulating LD size and access by lipases (Brasaemle 2007; Tansey et al. 2001), whilst Plin2/Adipophilin acts as the major LD coat protein that is especially important in the de novo formation of LDs and their maturation after release from the ER (Chang and Chan 2007; Bickel et al. 2009). In addition to these two basic members of the PAT/Perilipin family that are common to all animals, mammals have three additional members, Plin3-5, that act as regulators of LD formation/turnover (Wolins et al. 2006a, b) and may be capable of functionally substituting for Plin 1 (Sztalryd et al. 2006). A very basic overview of the regulation of LD formation/turnover in mammals is shown in Fig. 4.

Mammals also have an additional protein family, the Cidea group (cell death-inducing DNA fragmentation factor-like effector), which has a similar function to some Plin isoforms. One member of the Cidea group, Fsp27, promotes the formation of the large unilocular LDs that are characteristic of white adipocytes, and knockouts of the *fsp27* gene resulted in smaller LD size and increased rates of lipolysis (Puri and Czech 2008; Nishino et al. 2008). In general, Cidea-deficient mutants have similar lean phenotypes to Plin1 mutants and may be part of a redundant and therefore more resilient network of lipid regulation in mammals (Zhou et al. 2003; Christianson et al. 2010). In mice, the white adipocytes of Fsp27-deficient mutants also accumulated numerous small LDs and had elevated mitochondrial activity that is more characteristic of brown adipocytes (Toh et al. 2008). This implies that Fsp27 plays a role in promoting TAG biosynthesis and LD maturation directed towards the formation of large unilocular organelles that have a mainly storage function. In the absence of Fsp27 function, the default pathway, even in white adipocytes, appears to accumulate smaller LDs that can be more rapidly mobilized. In humans, Fsp27 is also an LD-bound protein that is associated with insulin sensitivity (Puri et al. 2007, 2008). Analysis of the proteome of LDs from rat hepatocytes revealed a novel protein termed 'Associated with LD protein 1', or ALD1 (Turró et al. 2006). As with many other LD proteins, ALD1 was initially found on the ER, but then became associated with nascent LDs where it appeared to be considerably more mobile than other LD protein components such as Plin2. The precise function of ALD1 has yet to be determined, but as the corresponding gene is only expressed in liver and kidney tissues, it may regulate specific forms of hepatic and nephritic LD behaviour that are distinct from LD behaviour in adipocytes.

Some additional roles of mammalian LDs

In addition to their well-known roles in neutral lipid biosynthesis, mammalian LDs can act as a second site (in addition to the ER) for the biosynthesis of the major membrane phospholipid, phosphatidylcholine (PC; Moessinger et al. 2011). Several other proteins that regulate various aspects of LD formation have recently been identified. These include PLD1 and ERK2 (Andersson et al. 2006), the BARS protein (Bartz et al. 2007b), Fsp27 (Puri et al. 2007), Prp19p (Cho et al. 2007) and FIT1/2 (Kadereit et al. 2008). Numerous lines of evidence point to an interaction of LDs with other subcellular compartments (Olofsson et al. 2008). Examples include the ER (Wu et al. 2000), ribosomes (Wan et al. 2007), endosomes (Liu et al. 2007), mitochondria (Jägerström et al. 2009) and peroxisomes (Binns et al. 2006). LDs also function as transient components of the membrane trafficking system, as short-term metabolic reservoirs for membrane acyl groups (Bartz et al. 2007a; Zehmer et al. 2009b), in signal transduction (Granneman and Moore 2008) and as rapidly turning over sites of protein storage and trafficking (Hodges and Wu 2010). Lipidomic studies are also consistent with roles for LDs in the trafficking of both phospholipids and ether lipids (Wu et al. 2000; Bartz et al. 2007a). As with *Drosophila* LDs (see "Lower animals"), mammalian LDs appear to act as storage sites for various hydrophobic proteins that may disrupt cell function if allowed to aggregate freely in the cytosol (Fujimoto and Parton 2010). Examples include apolipoprotein B-100 (Ohsaki et al. 2006, 2008) and α-synuclein (Cole et al. 2002; Webb et al. 2003).

The role of LDs in a range of caveolin-mediated intracellular transport processes has been demonstrated, and caveolins are a prominent and dynamic constituent of the mammalian LD proteome (Ostermeyer et al. 2001, 2004; Pol et al. 2001; Liu et al. 2004; Robenek et al. 2004; Martin and Parton 2005; Le Lay et al. 2009). Caveolins are named after caveolae, which are caveolin-enriched lipid raft-like structures found as 50- to 100-nm disk-shaped pits, or invaginations, in the plasma membrane of many mammalian cells. Caveolae give rise to a class of non-clathrin-coated vesicles that are involved in receptor-mediated endocytosis, cholesterol transport, TAG synthesis and signal transduction (Oh et al. 1998; Fujimoto et al. 2000, 2004; Nabi and Le 2003; Öst et al. 2005). Caveolin 2 is also associated with lipid rafts and ER-associated LDs

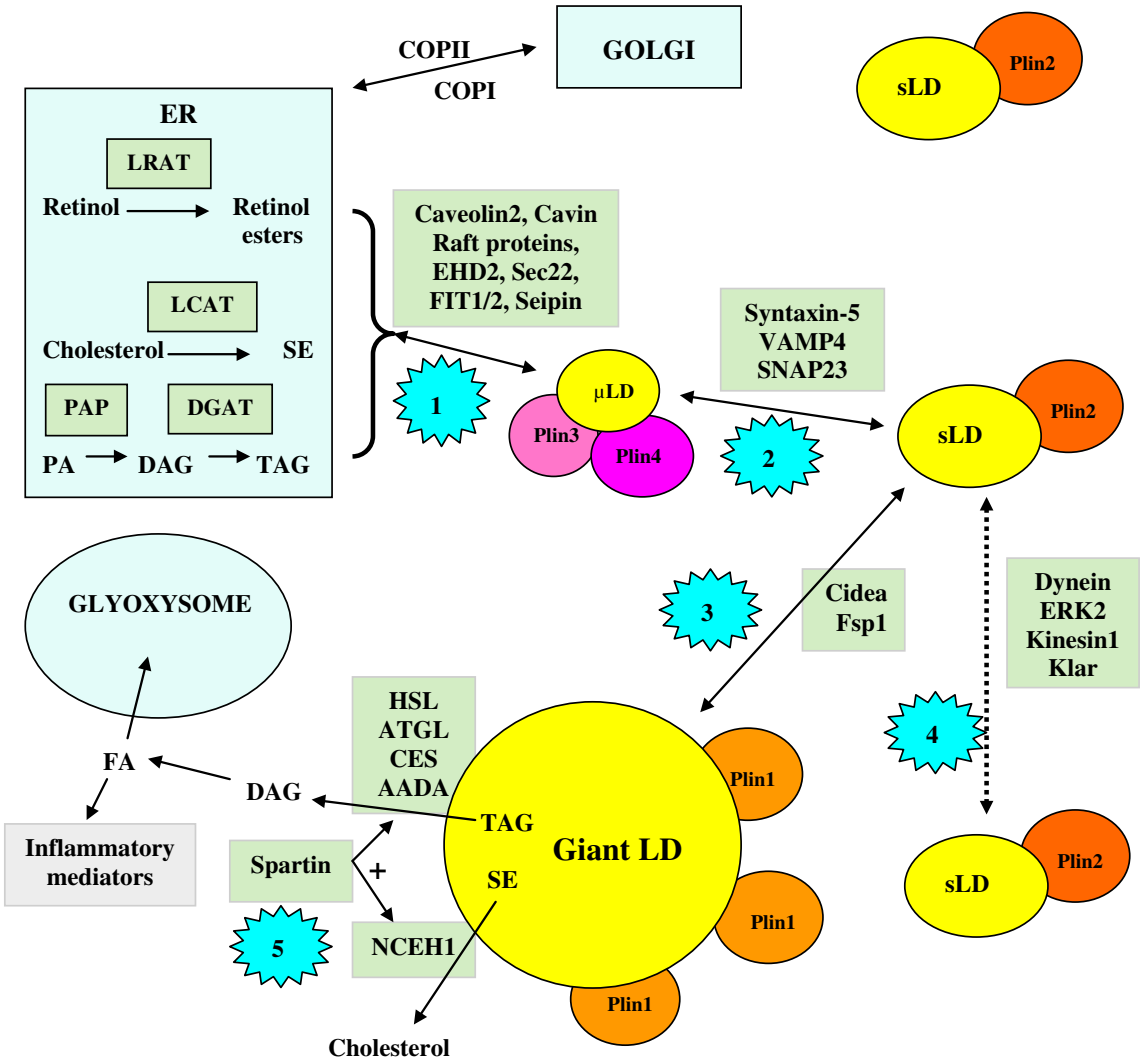

Fig. 4 Simplified overview of lipid droplet regulation in mammals. This is a brief summary of some of the major processes, components and organelles involved in the regulation of LD formation, maturation and turnover in mammalian cells. However, it should be stressed that these processes can vary considerably in different tissues and cell types, as well as being modified by numerous environmental factors including diet, stress, pathogen attack and ageing. The take-home message is that mammalian LD regulation is a highly complex process that plays a key role in cellular homeostasis and that its dysfunction is implicated in a host of pathological processes ranging from some forms of cancer to obesity and coronary heart disease. *1* Neutral lipid esters are released from the ER as µLDs in a process regulated by various proteins including caveolin2, seipin, cavin, etc. *2* In most cells, µLDs fuse to produce mature 0.5- to 2-µm LDs via a SNARE-mediated process. *3* In adipocytes, Cidea and Fsp1 stimulate small LDs to fuse further to giant 50- to 200-µm LDs. *4* In most cells, LDs are able to move along the cytoskeletal network via proteins such as Dynein and ERK2. *5* Mobilization of LDs is regulated via several hormones and protein factors such as Spartin

that bind σ-1 receptors; these receptors in turn bind lipidic neurosteroids and psychotropic drugs, including neuroleptics and cocaine, which also demonstrates the trafficking role of LDs (Hayashi and Su 2003). Caveolin trafficking is regulated by cholesterol and fatty acids and involves the Golgi, plasma membrane and LDs (Pol et al. 2005). Clearly, caveolins have a more complex and wider ranging role beyond acting as components of caveolae, and LDs are intimately involved in at least some of these additional functions (Parton and Simons 2007). Other proteins implicated in the trafficking of the aqueous-cored vesicles that are sometimes associated with LDs include numerous small G-proteins, EHD2, α-SNAP and Sec22 (Brasaemle et al. 2004; Liu et al. 2004), stomatin, which is additionally associated with lipid rafts and transport vesicles (Umlauf et al. 2004), and cavins, which are essential for the formation of caveolae as well as being implicated in LD function in mice (Liu et al. 2008), humans (Hayashi et al. 2009; Rajab et al. 2010) and cultured adipocytes (Aboulaich et al. 2006).

In addition to conventional TAG- or SE-rich LDs, some mammalian cells accumulate more specialized neutral lipids. For example, pigmented epithelial cells in the

mammalian retina and hepatic stellate cells both contain retinyl ester-enriched LDs termed retinosomes (Blaner et al. 2009; Orban et al. 2011). These esters are mobilized to replenish the visual chromophore, 11-*cis*-retinal, and their storage ensures proper visual function despite fluctuations in dietary vitamin A intake. Retinosomes also contain TAG, SE and typical LD-binding proteins such as Plin1-3 and Rab proteins such as CGI-58, whilst their formation is regulated by the enzyme lecithin:retinol acyltransferase (Orban et al. 2011).

Because mammalian LDs are dynamic and rapidly turning over organelles, the process of lipolysis in highly regulated via several different and overlapping signalling networks. It was originally believed that lipolysis of LD core lipids was catalyzed by a single enzyme, hormone-sensitive lipase (HSL). However, HSL knockouts were still capable of some lipolysis in white adipocytes (Osuga et al. 2000). In 2004, three laboratories independently characterized a novel TAG lipase, variously termed adipose triglyceride lipase (Zimmermann et al. 2004), desnutrin (Villena et al. 2004) and phospholipase A2ζ (Jenkins et al. 2004). Recent studies may provide evidence for yet more neutral lipid hydrolases (reviewed in Quiroga and Lehner 2011). These include some members of the carboxylesterase [Ces (mouse) or CES (human)] family [mouse Ces3/triglyceride hydrolase (TGH); its human ortholog CES1/CEH/TGH; mouse CesML1/TGH2; mouse Ces1/Es-x and Es22/egasyn; and arylacetamide deacetylase (AADA or AADAC) plus an AADA homolog KIAA1363, also named neutral cholesteryl ester hydrolase 1 (NCEH1) or arylacetamide deacetylase-like 1 (AADACL1)]. Puzzlingly, however, these enzymes are ER-associated glycoproteins with lumen-facing active sites, which is unexpected if their physiological substrates really are cytosolic LDs; more research is clearly needed to clarify their function. The process of lipolysis in adipocytes is also mediated by the GTPase, Rab18, which is recruited to the LD surface following insulin induction of a phosphatidylinositol 3-kinase (Pulido et al. 2011)

In mice, LD formation and lipolysis are both regulated by the ADP ribosylation factor (ARF)-like GTPase, ARFRP1, which is a molecular switch involved in Golgi function (Kahn et al. 2006). The deletion of this GTPase results in the activation of adipocyte TAG lipase and the disruption of LD maturation, leading to the virtual abolition of TAG accumulation in both white and brown adipocytes (Hommel et al. 2010). In addition to direct lipase-mediated breakdown, animal LDs can be degraded after incorporation, together with lysosomes, into so-called autolysosomes (Dong and Czaja 2011). The roles of lipases and other LD-associated proteins in LD mobilization as investigated using transgenic mice have recently been reviewed (Girousse and Langin 2011). Another example of global transcription factors regulating LD formation is the induction of the dehydrogenase/reductase, Dhrs3, by the stress-responsive transcription factor, p53 (Deisenroth et al. 2011). In addition to its roles in stress, p53 is now known to regulate cell metabolism under non-stressed conditions, and one of these roles is to induce Dhrs3 synthesis and accumulation at ER domains where TAG or retinol are being synthesized, which leads to the budding of Dhrs3-bound LDs.

In mammals, the enzymes that catalyze phosphatidic acid phosphatase activity (which produces DAG) are encoded by a family of genes originally named lipins. Because they control the cellular concentration of the bioactive lipids, phosphatidic acid and DAG, lipins are key regulatory enzymes of lipid metabolism and many signalling pathways. A role in neutral lipid homeostasis has been shown by the effects of lipin-1 overexpression in mice leading to obesity, whilst lipin-1 knockouts lack adipose tissue (Péterfy et al. 2001; Phan and Reue 2005). Lipin proteins are associated with several subcellular locations and can rapidly move within the cell where they may also act as transcriptional regulatory proteins in the nucleus (Gropler et al. 2009; Harris and Finck 2011). It has recently been reported that in human macrophages, lipin-1 is an LD-bound protein that regulates the activation of cytosolic group IVA phospholipase $A_2\alpha$, which is a pro-inflammatory enzyme that is involved in the release of LD-located arachidonic acid in order to form eicosanoid signalling mediators (Valdearcos et al. 2011). The wider roles of leukocyte LDs in inflammatory responses are reviewed by Bozza et al. (2009b, 2011), Bozza and Viola (2010) and Melo et al. (2011), whilst the nature of lipid signalling in disease has been reviewed by Wymann and Schneiter (2008).

The toxicity of some lipophilic environmental pollutants may involve LDs. For example, combustion-derived hydrocarbons, including carcinogenic components of cigarette smoke, are taken up into human bronchoepithelial cells and alveolar macrophages where they become localized on cytosolic LDs (Murphy et al. 2008). These toxins can also be taken up by adipocytes where the LDs could potentially act as long-term stores of carcinogenic toxins. If such accumulated toxins were released en masse once the LDs are mobilized, they could potentially have localized oncogenic effects.

Roles of LDs in disease

It is evident that mammalian cells have developed precise homeostatic mechanisms to regulate lipid uptake, synthesis, storage and usage (Horton et al. 2002; Coleman et al. 2000). In recent years, a great deal of mammalian lipid research has focused on metabolic dysfunctions whereby

either too much or too little lipid is stored in cytosolic LDs (Agarwal and Garg 2006). In humans, too little LD production results in lipodystrophy, which is a relatively rare and normally genetically related condition (Vigouroux et al. 2011). The most severe form of human lipodystrophy is Berardinelli–Seip congenital lipodystrophy, which is caused by mutations either in a TAG biosynthesis gene or in the LD-binding protein, seipin (Magré et al. 2001; Agarwal et al. 2002; Payne et al. 2008; Chen et al. 2009; Garg and Agarwal 2009). The study of seipin-defective mutations in humans and in various model organisms has revealed much useful information about the role of this protein (Fei et al. 2011a, b). For example, seipin deficiency leads to altered Δ9 fatty acid desaturase function as well as alterations in LD morphology (Boutet et al. 2009; Szymanski et al. 2007). It has been suggested that seipin may play a role in LD assembly by forming a collar at the ER–LD interface or by preventing LDs from being released into the ER lumen instead of the cytosol. However, further evidence is required to verify such a structural role because apparently normal cytosolic LDs were still observed in yeast and mammalian cells in the absence of Fld1p/seipin (Binns et al. 2010). Another type of lipodystrophy, autosomal dominant partial lipodystrophy, is caused by Plin1 deficiency, leading to a failure to accumulate LDs in white adipocytes (Gandotra et al. 2011). The human phenotype in this case is similar to that of Plin1 knockouts in some animal models (Grönke et al. 2007; Beller et al. 2010a).

In contrast to lipodystrophies where there is too little LD accumulation, the overproduction and ectopic appearance of LDs is associated with one of the most common metabolic malfunctions in human populations in the twenty-first century, namely obesity. Obesity (i.e. body mass index >30) affects 20–40% of the adult population of developed countries, and its prevalence has increased three to fourfold since the 1980s (Canoy and Buchan 2007). In terms of overall lipid mass, the bulk of LDs in normal adult mammals is present in white adipose tissue. White adipocytes differentiate from mesenchymal stem cells to preadipocytes and then to mature adipocytes, which are dominated by a single, giant unilocular LD that occupies over 90% of the cytosol (Rosen and MacDougald 2006). Obesity is characterized by the hyper-accumulation of LD-loaded white adipocytes and their ectopic proliferation in non-adipose tissues. Obesity-related lipid dysfunctions are implicated in several serious clinical conditions including metabolic syndrome, type 2 diabetes, hypertension, cardiovascular disease, osteoarthritis and cancer. For example, the LD-binding proteins, Plin2 and Plin5, are involved in ectopic LD accumulation in the skeletal muscle of both rats and humans (Minnaard et al. 2009), whilst Plin2 is also involved in diabetes (Mishra et al. 2004). The more general roles of LD dysfunction in some of these disease conditions have been reviewed by Le Lay and Dugail (2009), Meex et al. (2009) and Greenberg et al. (2011).

In order to understand the nature of obesity and to develop possible treatments, it is important to elucidate the molecular mechanisms of white adipocyte differentiation and the cellular dynamics (biogenesis, size, distribution and interaction with other organelles) of their cytosolic LDs. Equally, it is important to understand why some non-adipocyte cells, such as hepatocytes, cardiomyocytes and skeletal myocytes, appear to enter an adipocyte-like developmental programme and hyper-accumulate LDs. Such ectopic formation of LDs in non-adipocyte cells is correlated with metabolic syndrome and insulin resistance in most individuals in a process often termed ‘lipotoxicity’ (Unger 2002; van Herpen and Schrauwen-Hinderling 2008; Unger and Scherer 2010). Also, the hyper-accumulation of cholesterol ester-laden LDs in foam cells is a key stage in the development of arterial plaques and atherosclerosis. Indeed, some LD-associated proteins, such as Plin2, may have direct roles in atherogenesis (Larigauderie et al. 2004; Buers et al. 2011). Interestingly, however, there are a few exceptions to the rule that the presence of ectopic LDs lead to disease. For example, in the ‘athlete’s paradox’, highly trained elite athletes are able to accumulate high levels of TAG-rich LDs in skeletal muscle without developing dysfunctions such as insulin sensitivity (Goodpaster et al. 2001; Russell 2004).

Abnormalities in the function of LDs and/or LD-associated proteins have been found in several degenerative conditions including Parkinson’s and Altzheimer’s diseases (Bozza and Viola 2010). For example, the normally soluble Parkinson’s disease protein, σ-synuclein, becomes bound to LDs in affected patients, suggesting that relocation to LDs may be an early and important stage in the onset of this disease (Cole et al. 2002; Scherzer and Feany 2004). Another protein, called Nir2, which is essential for the proper function of the retina, can, upon phosphorylation, become relocated to LDs (Litvak et al. 2002). The resulting disruption of intracellular lipid trafficking may be involved in the degeneration of retinal function. The hereditary spastic paraplegias are a heterogeneous group of neurodegenerative disorders characterized by progressive lower limb spastic paralysis that are caused by mutations in a wide range of genes. One of these mutations that is also the cause of Troyer syndrome occurs in the gene encoding the recently characterized LD-associated protein, spartin. In normal individuals, spartin recruits and activates the ubiquitin ligase, AIP4, onto LDs, where it mediates their turnover by promoting ubiquitination of the major resident LD protein, Plin2 (Hooper et al. 2010). When spartin is absent or defective as in Troyer syndrome, LDs tend to accumulate to abnormal levels (Alberts and Rotin 2010). For some as yet unknown reason, in this case, the resulting

LD accumulation specifically affects neurons and leads to the progressive degeneration of motor neurons. In mice, a mutation of the *AUP1* gene results in a lethal neuromuscular disorder, and the gene product, ancient ubiquitous protein 1 (AUP1), is mostly bound to LDs where it binds E2 ubiquitin conjugases (Spandl et al. 2011). This demonstrates another direct molecular link between LDs and the ubiquitination machinery in mammalian cells.

Several forms of degenerative liver disease involve hepatic steatosis, or the hyper-accumulation of LDs in hepatocytes. In many cases, hepatic steatosis is induced by chronic alcohol intake (French 1989), and in a mouse model, enlarged Plin1-coated LDs were selectively induced by a high alcohol diet (Orlicky et al. 2011). Hepatic steatosis is also found in about half of all individuals who are chronically infected with the hepatitis C virus (Castera et al. 2005; Boulant et al. 2006, 2007, 2008). Although lipid accumulation was long thought of as a mere by-product of viral infection, recent work suggests that the viral life cycle is closely connected with liver lipid metabolism (Herker and Ott 2011). The major cause of LD accumulation in hepatocytes infected with the hepatitis C virus appears to be the action of one of the viral core proteins, which mimics the role of some of the endogenous Plin proteins of the host by directing the formation of LDs from the ER and then stabilizing the LDs by shielding them from lipolysis (Boulant et al. 2006; Roingeard et al. 2008). Recent work suggests that the LD production system of liver cells is essential for hepatitis C virus replication (Herker et al. 2010). The interferon-induced antiviral protein, viperin, which is induced by hepatitis C virus infection, is targeted to ER and LDs, possibly via an α-helical domain that competes for binding sites with the viral core protein (Hinson and Cresswell 2009). Other viral proteins are also able to subvert the LD trafficking system of their host cells in order to escape from the ER lumen (Ploegh 2007), whilst the dengue virus capsid protein hijacks LDs for viral particle formation (Samsa et al. 2009). Also, several human adenoviruses have been shown to act as stimulators of adipogenesis, both in vivo and in vitro (Whigham et al. 2005).

Abnormal functioning of LDs is associated with several forms of cancer (Menendez and Lupu 2007). A regular feature of some early stages of cancerous transformations is the appearance of putative microdomains of TAG within the lipid bilayer of cell membranes (Murphy 2001). Whilst aspects of these microdomains and their oncogenic significance remain controversial, they may have roles in abnormal LD formation and could be useful diagnostic tools for early-stage cancer detection (Khandelia et al. 2010; Delikatny et al. 2011). In neoplastic colon cancer cells, LDs proliferate and act as reservoirs of cyclooxygenase-2 and sites of prostaglandin-E2 synthesis (Accioly et al. 2008). In contrast, during the early stages of renal cell carcinoma, Plin2 (adipophilin) expression is upregulated, but then becomes gradually downregulated during subsequent stages of tumorigenesis and metastasis, with corresponding alterations in LD function (Yao et al. 2007). Finally, the major LD protein, Plin3 (TIP47), is involved in apoptotic and the differentiation processes of human epithelial cervical carcinoma cells (Than et al. 2003). In some forms of cancer, such as pancreatic and gastric cancers, and in some chronic infections, such as tuberculosis and AIDS, patients suffer from a progressive wasting condition called cachexia, which involves symptoms such as kidney and pulmonary disease and heart failure. Cachexia is the immediate cause of death in an estimated 15% of all cancer patients and appears to be the consequence of excessive lipase activity whereby LDs in adipocytes are broken down, resulting in the dramatic depletion in adipocyte size (Das et al. 2011). This is followed by muscle wasting due to fat and overall tissue loss that may be a secondary effect of lipid breakdown products such as cytokines, although this has yet to be tested experimentally (Arner 2011). The role of LDs in modulating the wide range of physiological and pathological stresses perceived by the ER network has been reviewed by Hapala et al. (2011).

Interactions of pathogens with LDs

As outlined in "Prokaryotes" and "Protists and fungi", the LDs of several pathogens that affect mammals are known to play important roles in their respective infection processes. However, as with the viruses discussed above, numerous microbial pathogens are also able to subvert the LD systems of their hosts for various purposes ranging from simply providing the pathogen with a food source to more sophisticated manipulations such as the induction of lipid mediators or the induction of specific sets of genes (Bozza et al. 2009a). Among the many examples of eukaryotic pathogens/parasites that disrupt LDs are the malarial agent, *P. falciparum*, which induces LD synthesis, storage and trafficking during its intraerythrocytic stages (Palacpac et al. 2004; Vielemeyer et al. 2004). The pathogen, *Trypanosoma cruzi*, which causes Chagas disease, induces LD formation in host macrophages and the generation of eicosenoids (Melo et al. 2003). Infections by several opportunist human pathogens that disrupt LD systems have become more common due the greater numbers of immunocompromised individuals with chronic HIV-1 infections. For example, the dimorphic fungus, *Histoplasma capsulatum*, causes a broad spectrum of diseases that can progress to life-threatening systemic infections. This fungus induces LD formation in host leukocytes by means of a secreted cell wall ß-glucan that interacts with host cell CD18, TLR2 and Dectin-1 receptors, resulting in

the generation of the signalling molecule, leukotriene B4 (Sorgi et al. 2009).

Several bacterial pathogens are also able to subvert the LD and lipid trafficking systems of their host cells. For example, many bacterial pathogens find it difficult to gain access to intracellular iron stores, but *M. tuberculosis* has evolved a strategy to accomplish this using host cell LDs as carriers (Luo et al. 2005). It is possible that mammalian LDs are normally involved in iron mobilization, in which case all the pathogen needs to do is to redirect iron-laden LDs towards the phagosome compartment in which it resides. The obligate intracellular pathogen *Chlamydia trachomatis*, is able to redirect endogenous cytosolic LDs within the cells of its host into the lumen of the parasitophorous vacuole (Cocchiaro et al. 2008). This is achieved by the secretion of Lda3 proteins from the pathogen into the host cell where they tag and capture cytosolic LDs that are then delivered to the inclusion lumen, from where they can be directly accessed by the pathogen for lipolysis. The related bacterium and cause of leprosy, *M. leprae*, is able to induce LD formation in macrophages via Toll-like receptors in order to generate eicosanoids required for pathogenesis (Mattos et al. 2010), whilst in host Schwann cells, the induction of LD formation may play a role in the survival of bacteria within phagosomes (Mattos et al. 2011).

One of the mechanisms of LD induction in macrophages during mycobacterial infections is via the activation of the ligand-dependent transcription factor, peroxisome proliferator-activated receptor γ (PPARγ; Almeida et al. 2009). This results in the proliferation of LDs and a reduced capacity for macrophages to attack the pathogen. In contrast, exposure to non-pathogenic *M. smegmatis* failed to trigger PPARγ expression in macrophages. On the other hand, if macrophage LD accumulation is decreased, the macrophages have an enhanced ability to kill infecting mycobacteria. This suggests that LDs play an important, albeit negative, role in the mounting of a successful anti-pathogen response in mammalian macrophages. PPARγ also plays a central role in adipocyte differentiation where it is regulated by various lipidic metabolites (Goto et al. 2011). Several other PPAR variants are involved in various aspects of normal LD function, such as PPAR∂ as a sensor of very low-density lipoproteins in macrophages (Chawla et al. 2003) and PPARα as an inducer of TAG and Plin2 protein formation in hepatocytes (Edvardsson et al. 2006).

In summary, mammalian cytosolic LDs have now emerged as integral components that are involved in an ever-growing list of subcellular processes in both health and disease. The correct functioning of LDs is crucial to many aspects of constitutive cellular homeostasis, and they often also play specialized roles in particular tissues or organs, ranging from the retina to the lung. However, one of the most interesting aspects of LD studies in recent years relates to the central roles that these organelles appear to play in several serious disease and degenerative conditions in humans, including those involved in major public health issues such as obesity/metabolic syndrome and cardiovascular disease. As discussed in previous sections, research in non-mammalian model systems has greatly improved our understanding of LD behaviour under a range of normal and pathological conditions. In the future, it will be important for mammalian LD researchers to be aware of and exploit the immense potential of these model systems to underpin further advances in LD-related studies in humans.

Mechanisms for the biogenesis and maturation of LDs

Many aspects of fundamental cellular processes, such as cell division and protein synthesis, are highly conserved in biological organisms, and this probably applies to such a widespread and important phenomenon as the formation and maturation of LDs. The physical processes involved in LD formation and release from cell membranes, and the continuing exchange of components between mature LDs and bilayer membranes, have yet to be explained in detail. In particular, there are very few thermodynamically rigorous models to account for how rapidly diffusing neutral lipid molecules such as TAGs and SEs are constrained to form swellings within membrane bilayers (Zanghellini et al. 2010a, b). Nor are there satisfactory explanations of how nascent droplets are able to bud off from or reattach to intact bilayer membranes. Although most textbooks and reviews (including those of the author) tend to depict the accumulation of TAGs as occurring in bulges or blisters within the ER bilayer, such structures are unlikely to arise spontaneously in a conventional planar bilayer membrane. This is because: (1) newly formed TAG/SE molecules would tend to disperse rapidly within the lateral plane of the bilayer and (2) the tight curvature around a small TAG/SE blister is thermodynamically unfavourable for most bilayer lipids, and especially the major ER lipid, PC. Therefore, how is it possible for localized TAG/SE inclusions to form inside a phospholipid bilayer? There are several possible mechanisms that might operate in LD-forming organisms, two of which are highlighted here.

Firstly, nascent TAG/SE molecules may be constrained from lateral diffusion by physical barriers, such as transmembrane proteins that can effectively pen in the TAGs so that they forced to form a bulge in the bilayer (see Fig. 5). Lipid rafts provide a mechanism to generate lateral heterogeneity in membranes (Jacobson et al. 2007), and

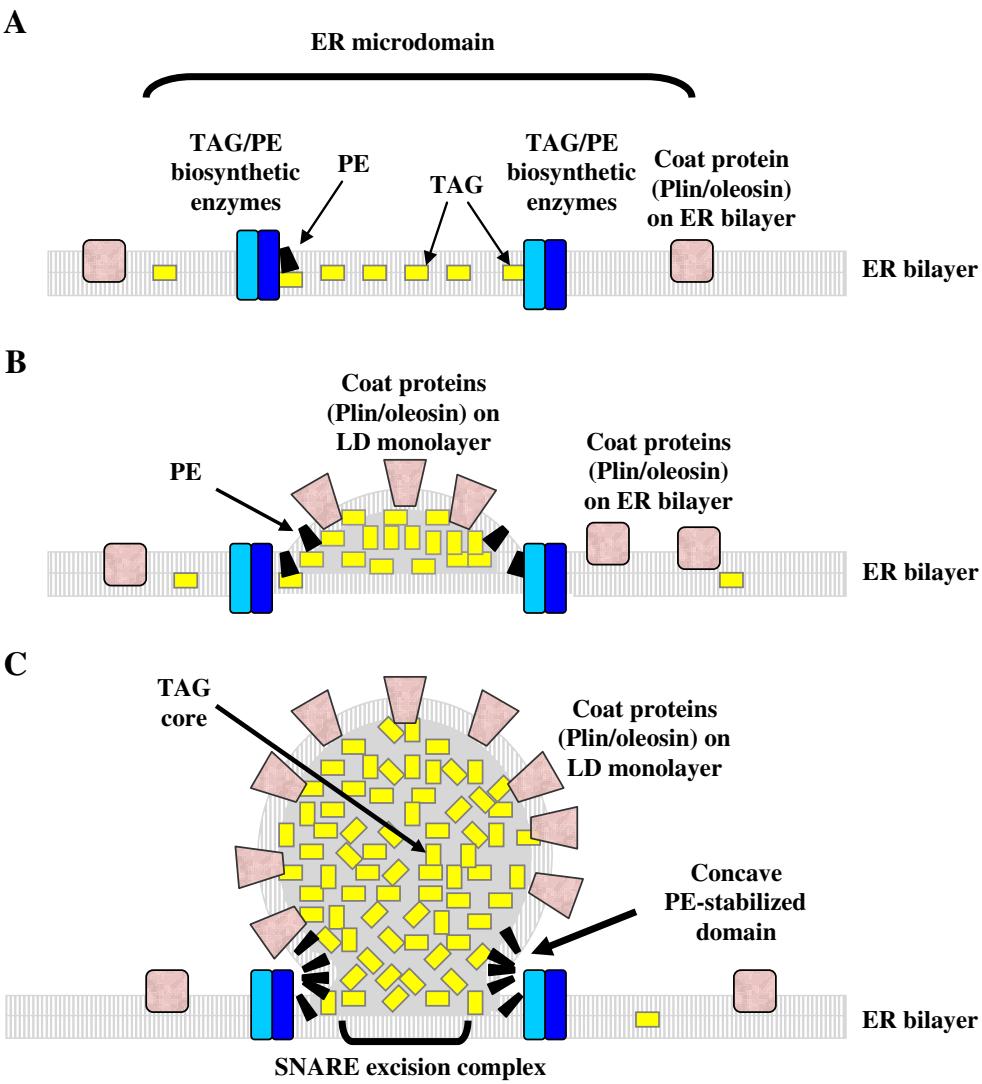

Fig. 5 Model of lipid droplet formation on the ER membrane. **a** LD formation occurs in discrete ER microdomains that are enriched in enzymes of neutral lipid ester (e.g. TAG) and non-planar bilayer lipid (e.g. PE) biosynthesis, plus LD coat proteins such as Plin or oleosin. Lateral diffusion of the TAG and PE products is physically restricted by a ring of transbilayer proteins around their site of formation. **b** The accumulating TAG (*yellow rectangles*) within the ER bilayer is forced into a cytosol-facing bulge that is stabilized at its concave neck by localized domains enriched in PE (*black wedges*) and by coat proteins that are recruited into the bulge, possibly thanks to the favourable free energy transition conferred by moving from a monolayer (*pink rectangles*) to a bilayer (*pink wedges*) environment. **c** As more TAG enters the bulge and its neck is increasingly constrained, it begins to assume a spherical shape. The final excision of the neck to release a nascent μLD may be facilitated by a SNARE complex, as shown in Fig. 7

specific transmembrane proteins could be recruited into localized lipid raft domains that are enriched in TAG/SE biosynthetic enzymes. It is known that LD formation occurs on membrane microdomains in both prokaryotes and eukaryotes, and these are probably related to lipid rafts (Browman et al. 2007). For example, some lipid raft proteins with characteristic SPFH (stomatin/prohibitin/flotillin/HflK) domains, such as Flotillin-1 and Flotillin-2 and stomatin, co-localize on various cellular membranes and on LDs (Rajendran et al. 2007). In particular, Flotillin-1 appears to specifically associate with nascent LDs, and it has been proposed that it could induce vesiculation at the ER or plasma membrane following loading of the bilayer with neutral lipids. Flotillin-1 has recently been described as a determinant of a clathrin-independent endocytic pathway in mammalian cells, which implicates this protein in vesicular trafficking (Glebov et al. 2006), whilst its expression in insect cells can induce the formation of caveolae-like vesicles (Volonte et al. 1999).

Secondly, the increased membrane curvature around growing TAG/SE blisters can be stabilized by phospholipid demixing (Mukherjee and Maxfield 2004; Zanghellini et al. 2010a, b; van Meer et al. 2008; van Meer and de Kroon 2011). Simulations using grained molecular dynamics have shown that lipid bilayers can accommodate 17-nm diameter TAG-rich domains, and these may be the precursors of LDs as well as providing diagnostic evidence of early malignancy in certain cancers (Khandelia et al. 2010). The demixing of bilayer phospholipids can be generated by the selective biosynthesis of cone-shaped lipids in TAG/SE-rich regions of the ER. Such lipids have headgroups with relatively small cross-sectional areas and larger acyl chains. The importance of conical lipids in the stabilization of curved bilayer regions has been demonstrated for plant membrane systems (Murphy 1982). Two of the most common conical lipids capable of stabilizing regions of highly negative membrane curvature are phosphatidylethanolamine (PE) and DAG (Mukherjee and Maxfield 2004;

Dowhan et al. 2008). In addition, signalling events can lead to the localized accumulation of highly charged lipids such as phosphorylated phosphoinositides or phosphatidic acid (PA) and lyso-PA on one bilayer leaflet (Jenkins and Frohman 2005). Such lipids can themselves induce curvature and/or recruit specific proteins to such localized domains that then influence curvature. Current concepts of the energetic implications of membrane curvature and the roles of lipids and proteins in generating such curvature are based on the theory of membrane bending elasticity as proposed originally by Helfrich (1973), and discussed and extended more recently by Zimmerberg and Kozlov (2006).

Recently, it has been shown that in mammalian systems, PE may play a key role in stabilizing the tightly curved TAG blisters and micro-LDs required for LD formation (Hörl et al. 2011). Although the most common lipid component of the ER membrane is PC, during LD formation, there is an upregulation of enzymes involved in the conversion of PC to PE (Vance and Vance 2008). The localized high concentration of PE in the vicinity of TAG formation may stabilize TAG blisters within the ER bilayer and may also promote their evagination as micro-LDs. These results are consistent with the observation in plants that plastid LDs, or plastoglobules, are associated with the tightly curved margins of thylakoid membranes (Austin et al. 2006). We have previously shown that thylakoid margins are enriched in and stabilized by cone-shaped monogalactolipids (Murphy and Woodrow 1983). As shown in Fig. 5, the directionality of LD release could be promoted by the selective accumulation of PE on the cytosolic monolayer of TAG/SE-producing ER domains. Providing the TAG/SE and PE molecules in such domains were constrained from lateral diffusion within the bilayer, as discussed above, the presence of localized PE clusters could facilitate an outward, cytosol-facing bulge in the ER that could then be stabilized by the recruitment of structural LD-binding proteins such as Plin2 (in Unikonts) or oleosin (in plants).

This process would result in the formation of a large cytosol-facing bulge enriched in neutral lipids and enclosed/stabilized by a phospholipid/protein coat. It is possible that in some cells, these LD bulges stay attached to the ER via a narrow neck stabilized by lipids such as PE and specialized proteins such as SNAREs. This would enable their contents to remain in dynamic equilibrium with metabolite pools within the ER. For example, cytosolic LDs in the moss, *P. patens*, appear to maintain physical continuity with the ER, whereas the LDs in the seeds of higher plants lose their connection with the ER as they mature (Huang et al. 2009). As shown in Fig. 6 and reported by us previously (Piffanelli et al. 1997), there are very close associations between LDs and ER membranes in certain plant cells, and in many cases, LDs appear to be partially or completely encircled by closely appressed ER sheets or tubules. Similar findings have been reported from other plant and animal systems (Fisher et al. 1968; Jensen et al. 1968; Wetzel and Jensen 1992; Targett-Adams et al. 2003; Robenek et al. 2005a, b; Bozza et al. 2007). In the analogous process of plastoglobule formation in plant plastids, there is evidence that at least some plastoglobules remain in direct contact with the thylakoid membranes from which they were formed (Austin et al. 2006).

The process of LD formation is separate from TAG biosynthesis and requires the presence of additional proteins. The best known of these are LD coat proteins such as Plin2, oleosin or phasins, but other ER-resident proteins are now known to be essential to LD formation and release. One example is the family of fat-inducing transcript (FIT) proteins found in most, and perhaps all, Unikonts (Kadereit et al. 2008). Confusingly, these FIT proteins are completely unrelated to the similarly named fungal LD proteins where FIT stands for fat storage-inducing transmembrane (Nguyen et al. 2011). We (Abell et al. 1997; Sarmiento et al. 1997) and others (Abell et al. 2002, 2004) have previously suggested that LD-associated proteins might facilitate LD formation by assuming a lower energy conformation on a budding LD monolayer compared to their conformation on or within the cytosolic face of the ER bilayer. This would provide a thermodynamic driver for the partitioning of such proteins onto the LD surface and could facilitate the formation of a cytosol-facing bulge in the ER bilayer. Such a tightly curved bulge in the outer face of the ER bilayer would be additionally stabilized by the demixing of conical phospholipids, as discussed above. Meanwhile, the lateral escape of neutral lipids from the incipient LD would be further constrained by the presence of transmembrane proteins, possibly including seipin (Binns et al. 2010), which could form an ever-diminishing collar around the neck of the LD (see Fig. 5).

Whilst some LDs remain physically and/or functionally connected with the ER, others (perhaps the majority in most cells) may be released into the cytosol as microdroplets (μLD) of about 50- to 60-nm diameter, although a recent theoretical model predicts that the earliest nascent μLDs may be as small as 12 nm in diameter (Zanghellini et al. 2010a, b). It seems likely that a similar process occurs during PHA granule formation in prokaryotes, although in this case the granules bud off from the plasma membrane (Wältermann and Steinbüchel 2005). These various types of μLD probably then undergo fusion via highly regulated processes in order to produce the small LDs (sLD) of about 500- to 2,000-nm diameter, as found in the mature LD populations of most cell types in most organisms. In mammalian cells, it was observed that spontaneous fusion of LDs was relatively slow under normal growth conditions, but was promoted by several pharmacological fusogenic agents (Murphy et al. 2010).

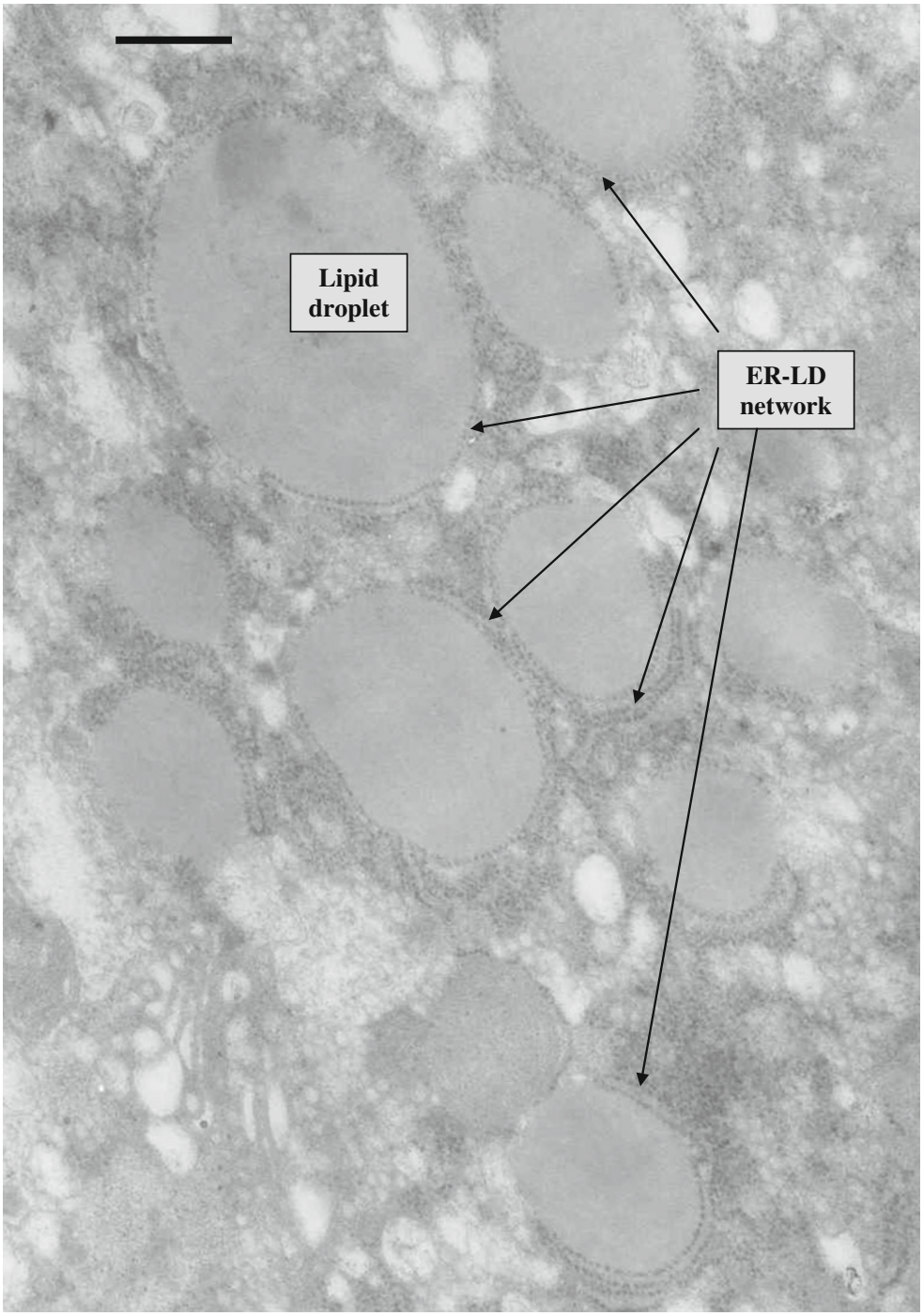

Fig. 6 ER–lipid droplet associations in plants. Cytosolic lipid droplets in developing microspores in the plant, *Brassica napus*. Note the close association between the lipid droplets and the ER membrane system, which in many cases appears to enfold the droplets. Similar ER–LD associations are found in animals and other eukaryotes. *Bar*, 0.5 μm

When two spheres fuse, the surface area/volume ratio of the new larger sphere is smaller than the sum of the two original spheres (see Fig. 7). As the internal volume of fused LD spheres is conserved, some of the surface PL and/or protein components (about 20%) must be removed rapidly in order to stabilize each newly enlarged LD (Ohsaki et al. 2009; Murphy et al. 2010). It is estimated that the surface PL content of a mature adipocyte giant unilocular LD (gLD) is only one tenth of the PL content of all the μLDs that originally fused to produce it (Zanghellini et al. 2010a, b). During LD growth, membrane curvature decreases substantially, and, as expected if PE tends to stabilize μLDs, LD maturation has been found to be correlated with the conversion of PE back to PC (Hörl et al. 2011). Whilst many models of LD maturation involve fusion of smaller LDs and the consequent generation of surplus phospholipids and coat proteins (Boström et al. 2005, 2007), other models posit the growth of LDs via the gradual accretion of neutral lipids, as occurs in caveolae (Uittenbogaard et al. 2002; Kuerschner et al. 2008; Yen et

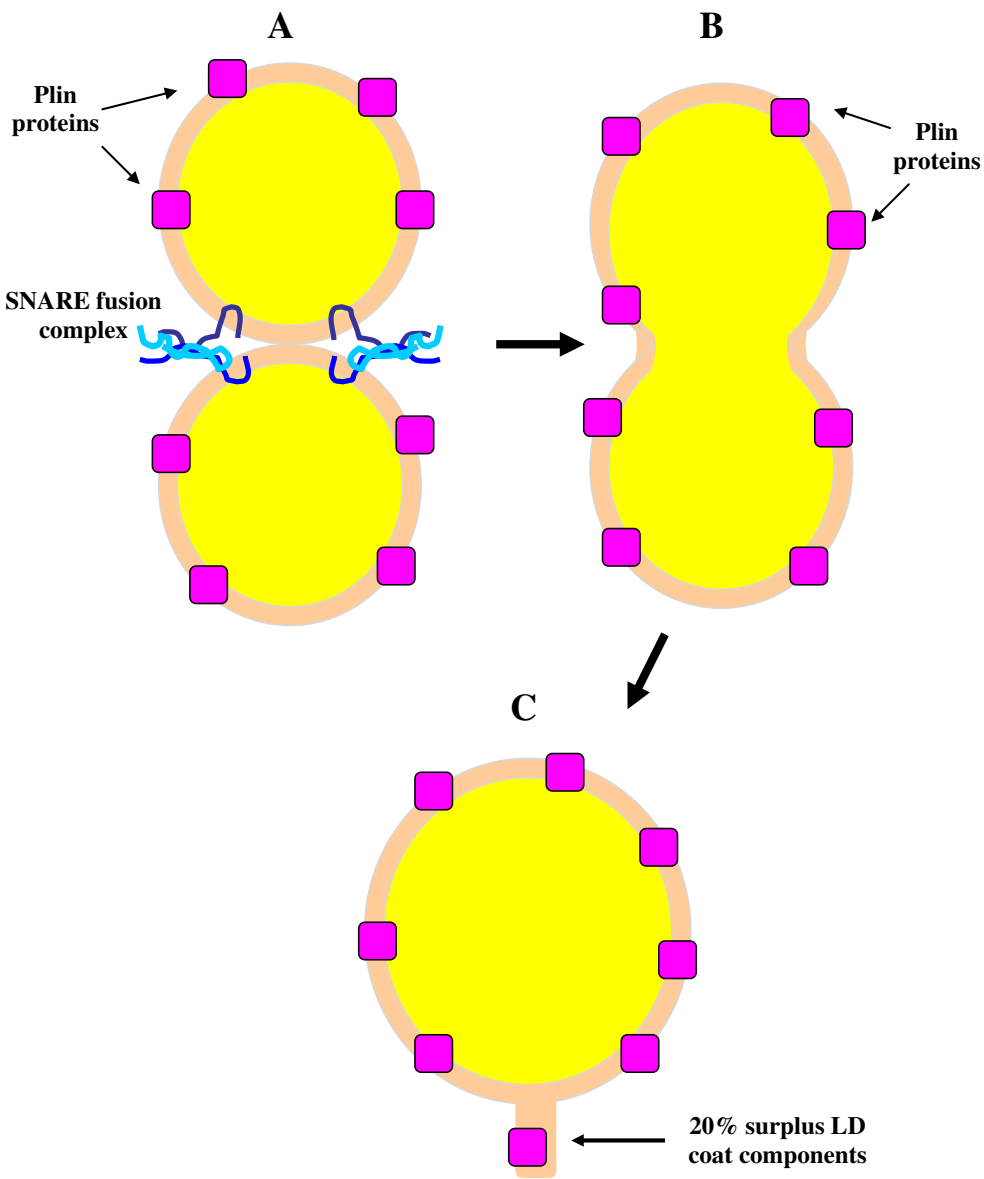

Fig. 7 Model for lipid droplet fusion in mammals. **a** In this model, two equal-sized lipid droplets become tethered together via a SNARE fusion complex consisting of a four-helix bundle formed between Syntaxin-5, SNAP23 and VAMP4. **b** The SNARE complex forces the two lipid monolayers together until they fuse, which enables the neutral lipid cores to combine into a single larger droplet. The resulting droplet will be about 26% larger in diameter than each original droplet and will have a 20% excess of phospholipid/protein surface area that will require removal or recycling. See text for further details. Adapted from Olofsson et al. (2009)

al. 2008; Cheng et al. 2009). In contrast with LD fusion, the accretion mechanism would require the recruitment of additional PLs and LD coat proteins in order to stabilize the growing LDs (Fujimoto et al. 2008).

In most mammalian cells, the accumulation of a small number of sLDs appears to be the default mechanism. However, in a very small number of cell types, most notably white adipocytes, small numbers of sLDs are not the mature state. Instead, sLDs continue to fuse with each other until the cell is almost entirely filled with a single giant unilocular LD. This process is mediated by LD maturation genes, such as *fsp27*, and lesions in such genes can result in the failure of sLDs to fuse into gLD in white adipocytes (Le Lay and Dugail 2009). In contrast, the default LD maturation pathway in some cells in *Drosophila* appears to be the formation of a unilocular gLD, but this is normally prevented by the action of Plin1 to promote lipolysis (Beller et al. 2010a). Therefore, Plin1 deficiency in *Drosophila* often leads to a unilocular LD phenotype, whereas in humans, it results in a complete failure to accumulate LDs in white adipocytes (Gandotra et al. 2011). In addition to regulatory proteins such as Fsp27, LD fusion in adipocytes involves microtubule and SNARE proteins, and the latter are implicated in insulin sensitivity (Boström et al. 2005, 2007). These proteins, which also play roles in vesicle budding and fusion, probably facilitate the fusion of LDs. In mammals, LD fusion may also be promoted by the recruitment by Arf1-type GTPases of phospholipase D, resulting in the generation of localized accumulations of the non-planar bilayer lipid, PA (Nakamura et al. 2005; Andersson et al. 2006; Walther and Farese 2009).

The importance of LD fusion can be appreciated from the calculation that it requires about 1,000 μLDs to fuse in order to produce a typical mature unilocular LD in a white

adipocyte. It has been proposed that LD fusion occurs via a simplified version of SNARE-mediated bilayer vesicle fusion (Boström et al. 2007 et al.; Olofsson et al. 2009). According to this model, two small LDs approach and are tethered together via a SNARE complex that consists of a four-helix bundle between Syntaxin-5, SNAP23 and VAMP4. As shown in Fig. 7, this SNARE complex pulls the two adjacent monolayers towards each other, eventually forcing them to fuse into a single monolayer that now encloses the combined contents of both original LDs. Following LD fusion, the four-helix bundle is recognized by α-SNAP, which, together with the ATPase, NSF, unwraps the bundle, thus enabling new fusions to be initiated. It is likely that additional proteins, such as members of the Plin family, then either stabilize the new enlarged LD or facilitate its continued fusion to create even larger LDs. As noted above, individual mammalian cells can contain several size classes of LD, each stabilized and regulated by different groups of Plin proteins. It is likely that these LD size populations are dynamic, shifting towards larger LD sizes when lipid storage is favoured and to smaller sizes when LD turnover and mobilization are required. The disruption of this dynamic process appears to be a central event in the interrelated pathologies of metabolic syndrome, obesity and insulin sensitivity.

Concluding remarks

The past decade has witnessed a transformation in our knowledge of the roles of LDs in cellular function across the full range of biological organisms. We are beginning to uncover the true extent of the function of LDs in highly dynamic short-term processes such as signalling, as well as in longer-term processes such as energy storage. In the higher multicellular eukaryotes, LDs also have roles in developmental processes, environmental responses, pathogenesis and ageing that are often manifest at the whole organism level. In particular, LD dysfunction in humans is now implicated in some of the most serious contemporary public health concerns, including obesity, insulin-resistant diabetes and coronary heart disease. In the coming years, the elucidation of the precise roles of LDs in health and disease in a range of model organisms, from yeast to mice, will doubtless greatly extend our understanding of human LD dysfunction and hopefully will enable new methods of prevention and treatment to be devised.

There has also been much progress in the harnessing of LDs as feedstocks for the production of renewable biofuels, e.g. in microalgae, and a broad range of renewable and biodegradable industrial materials, including biopolymers and oleochemicals. Finally, the fats and oils derived from plant and animal LDs provide the second most important source of dietary calories to human societies across the world. The task of increasing global food supplies has become increasingly urgent in this era of food insecurity and climate change. In the future, the emerging ability to engineer ectopic LD formation in little-used crop tissues, such as leaves and roots, has the potential to greatly increase our supply of lipids that could be used for food, fuel and chemical production. In short, the study of LDs has now emerged fully from its previous 'Cinderella' status to become one of the central themes of modern cell biology. Moreover, continuing advances in analytic techniques and genomics give the prospect of continued advances in this exciting area for many years to come.

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