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# The characteristics and potential applications of structural lipid droplet proteins in plants

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ABSTRACT

Plant cytosolic lipid droplets are storage organelles that accumulate hydrophobic molecules. They are found in many tissues and their general structure includes an outer lipid monolayer with integral and associated proteins surrounding a hydrophobic core. Two distinct types can be distinguished, which we define here as oleosin-based lipid droplets (OLDs) and non-oleosin-based lipid droplets (NOLDs). OLDs are the best characterized lipid droplets in plants. They are primarily restricted to seeds and other germinative tissues, their surface is covered with oleosin-family proteins to maintain stability, they store triacylglycerols (TAGs) and they are used as a source of energy (and possibly signaling molecules) during the germination of seeds and pollen. Less is known about NOLDs. They are more abundant than OLDs and are distributed in many tissues, they accumulate not only TAGs but also other hydrophobic molecules such as natural rubber, and the structural proteins that stabilize them are unrelated to oleosins. In many species these proteins are members of the rubber elongation factor superfamily. NOLDs are not typically used for energy storage but instead accumulate hydrophobic compounds required for environmental interactions such as pathogen defense. There are many potential applications of NOLDs including the engineering of lipid production in plants and the generation of artificial oil bodies.

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## 1. Introduction

Lipid droplets are cellular storage organelles that sequester neutral lipids from the cytoplasmic environment. Plant lipid droplets have mainly been studied in seeds, where they are also known as oil bodies or oleosomes. This tissue is rich in lipids, predominantly triacylglycerols (TAGs), which are broken down during germination to provide an initial source of energy for the growing seedling (Huang, 1992, 1996). The major structural proteins in seed-derived lipid droplets are oleosins, complemented by a smaller quantity of caleosins, which are necessary to maintain the structural stability of these organelles (Chapman et al., 2012; Murphy, 2001; Tzen and Huang, 1992; Tzen, 2012). Less attention has been paid to lipid droplets in other tissues even though they are present in most cells (Lersten et al., 2006; Murphy, 2001). We can therefore distinguish two distinct types of lipid bodies, which we define here as the oleosin-based lipid droplets (OLDs) predominantly found in seeds and the non-oleosin-based lipid droplets (NOLDs), which are also found in many other tissues. Recent publications have shown that the structural proteins in NOLDs are not related to oleosins, and in the lipid-enriched tissues of avocado (*Persea americana*), dandelion (*Taraxacum brevicorniculatum*) and the rubber tree (*Hevea brasiliensis*) they have been shown instead to belong to the rubber elongation factor (REF) superfamily (Berthelot et al., 2014b; Dennis and Light, 1989; Horn et al., 2013; Oh et al., 1999; Schmidt et al., 2010a).

*Abbreviations:* AOB, artificial oil body; CPT, *cis*-prenyltransferase; CsPAT, *Cucumis sativus* patatin-like protein; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; HLL, *Hevea brasiliensis* latex lectin; HSD, hydroxysteroid hydrogenase; LDAP, lipid droplet-associated protein; LEC2, leafy cotyledon 2; MLDP, major lipid droplet protein; MuSI, multiple stress responsible gene I; NOLD, non-oleosin-based lipid droplet; OBAB1, oil body-associated protein 1; OLD, oleosin-based lipid droplet; PaGHS, *Parthenium argentatum* small rubber particle protein homolog; PDAT1, phospholipid:diacylglycerol acyltransferase; REF, rubber elongation factor; SRP, stress-related protein; SRPP, small rubber particle protein; TAG, triacyglycerol.

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Lipid droplets have been considered as potential biotechnology-based production platforms (Bhatla et al., 2010; David et al., 2013; Durrett et al., 2008; Roberts et al., 2008). Approaches include the production of specific hydrophobic molecules or recombinant proteins in manipulated or artificial lipid droplets, as well as the production of biofuels in lipid-rich plant tissues as an alternative energy source. In the latter case, lipid-rich seeds from crops such as sunflower (*Helianthus annuus*) and rapeseed (*Brassica napus*) could be used as an energy source, but they are also used in the food industry and new biotechnology-based products should not compete with the food supply (Ohlrogge and Chapman, 2011). An alternative approach is to enrich lipids in vegetative tissues such as leaves, which are waste products in many crops. Understanding the molecular basis of lipid droplet formation should allow the development of tailored lipid droplets in vegetative tissues either for industrial applications or as an energy source. Proteins required for the stability and activity of NOLDs have been identified recently. In this review, we focus on lipid droplet proteins by comparing the protein composition and structure of the two classes of lipid droplets and how these properties influence their potential applications.

## 2. There are two broad classes of lipid droplets in plants

Here we define lipid droplets as cellular organelles with a lipid core surrounded by a phospholipid monolayer containing integral and/or associated proteins. The characteristic lipid droplet structure allows emulsification, so that hydrophobic lipids can be stored in the hydrophilic cytoplasm (Thiam et al., 2013). The most prominent components are TAGs, which are important storage lipids needed for seed development and energy mobilization (Bewley and Black, 1994; Lung and Weselake, 2006; Stymne and Stobart, 1987). However, other hydrophobic molecules are stored in lipid droplets including natural rubber, sterols and phytoanticipines, which are used by plants to defend against pathogens and herbivores (Dussourd and Eisner, 1987; Dyas and Goad, 1994; Murphy, 2001; Shimada et al., 2014). Natural rubber is stored in specialized lipid droplets known as rubber particles, although their structure is similar to the lipid droplets that store TAGs (Cornish, 2001).

Structural proteins including oleosins and REF superfamily proteins are associated with the lipid droplet surface to maintain structural integrity while allowing dynamic functions (Frandsen et al., 2001; Hillebrand et al., 2012; Leprince et al., 1997; Peng et al., 2003). Lipid droplets may also include additional proteins, such as enzymes responsible for the synthesis or degradation of the storage molecules, *e.g. cis*-prenyltransferases (CPTs), diacylglycerol acyltransferases (DGATs) and lipoxygenases, proteins required for lipid droplet biosynthesis, and also signaling proteins (Jolivet et al., 2004, 2013; Feussner et al., 2001; Post et al., 2012; Valencia-Turcotte and Rodríguez-Sotres, 2001; Yadav and Bhatla, 2011).

We can divide lipid droplets into two groups based on their tissue specificity, structure and function, according to Chapman et al. (2012). The first group is the oleosin-based lipid droplets (OLDs), which are often found in seeds but not strictly restricted to this tissue. Their unique defining feature is that they require the presence of oleosin-family proteins for their structural integrity. Oleosins are co-translationally translocated into the sections of the endoplasmic reticulum (ER) that form lipid droplets. The second group is found in many tissues and is not preferentially located in seeds, and its unique defining feature is that the lipid droplets form in the absence of oleosins. These non-oleosin-based lipid droplets (NOLDs) are not well characterized, and include rubber particles and the oil bodies found in avocado fruits.

There is one further class of specialized lipid droplets known as plastoglobuli, which store hydrophobic molecules and proteins associated with photosynthesis (Murphy, 2001; Smith et al., 2000; Tevini and Steinmüller, 1985). Because these are only found in plastids and do not appear to store metabolites, they are not discussed further in this article.

## 3. The structure of plant lipid droplets facilitates their dynamic behavior

Most research on lipid droplets has focused on OLDs in seeds so we consider this topic first. However, the biogenesis of all lipid droplets appears to be similar and the process is illustrated for both droplet types in Fig. 1. Lipid droplets originate in the ER, not only in plants but also in mammals and yeast (Athenstaedt and Daum, 2006; Craig and Staehelin, 1988; Farese and Walther, 2009; Krahmer et al., 2009; Shockey et al., 2006; Zweytick et al., 2000). Typically, the hydrophobic storage molecules are synthesized in distinct regions of the ER and are sequestered between the two leaflets of the ER membrane along with the corresponding enzymes and structural proteins (Athenstaedt and Daum, 2006; Lacey and Hills, 1996; Lacey et al., 1999; Martin and Parton, 2006; Zweytick et al., 2000). This promotes the formation of a bud in the ER membrane, although it is unclear whether this is induced by the activity of certain proteins or simply reflects the accumulation of hydrophobic molecules (Hsieh and Huang, 2004; Lacey et al., 1999; Ross et al., 1993; Sarmiento et al., 1997). When the lipid pool reaches a certain threshold, a droplet buds from the membrane (Murphy, 2001; Wanner and Theimer, 1978; Wanner et al., 1981). The mechanism of lipid droplet growth is unknown and may differ among different types of lipid droplets and different types of cells (Chapman et al., 2012; Heneen et al., 2008; Miquel et al., 2014). Lipid droplets may grow to their final size while still attached to the ER membrane so no further expansion occurs following release, or they may continue depositing additional lipids into the mature droplet core after budding, as has been shown *in vitro* for rubber particles (Archer et al., 1963; Asawatreratanakul et al., 2003). A third process of maturation involves the fusion of small oil bodies, as observed in rapeseed and Arabidopsis (*Arabidopsis thaliana*) seeds (Frandsen et al., 2001; Huang, 1996; Miquel et al., 2014; Murphy and Vance, 1999; Sarmiento et al., 1997).

TAG synthesis in the ER is closely linked to the formation of OLDs in a spatiotemporal manner. It has been shown that enzymes required for TAG biosynthesis are localized in the ER, *e.g.* the Kennedy pathway takes place in the ER lumen (Athenstaedt and Daum, 2006; Krahmer et al., 2009; Ohlrogge and Browse, 1995). The enzymes that convert glycerol-3-phosphate and plastid-derived activated fatty acids into TAGs are also found in the ER, namely glycerol-3-phosphate acyltransferase, 1-acylglycerol-3-phosphate acyltransferase and phosphatidate phosphatase (Athenstaedt and Daum, 2006; Krahmer et al., 2009). The last step is catalyzed by DGAT and this generates storage TAGs (Lung and Weselake, 2006; Ohlrogge and Browse, 1995; Zhang et al., 2009). DGAT is found in the ER but also on the surface of OLDs already detached from the ER (Jolivet et al., 2013; Valencia-Turcotte and Rodríguez-Sotres, 2001). These findings indicate that the synthesis of lipids is restricted to certain ER regions thus enhancing the efficiency of synthesis by ensuring the co-localization of the necessary proteins (Chapman et al., 2012; Lacey et al., 1999; Shockey et al., 2006).

Lipid droplets that store TAGs in seeds are incredibly stable, but the TAGs must be degraded to provide energy during germination and beyond, so the lipid droplets are targeted by lipolytic enzymes such as patatin-like TAG lipases, phospholipases, lipases and lipoxygenases (Feussner et al., 2001; Huang, 1992, 1996; Zienkiewicz et al., 2013). Furthermore, seed lipases are localized on the lipid droplets of Arabidopsis seeds and affect the oil content of Barbados nut (*Jatropha curcas*) seeds (Eastmond, 2006; Hills and Beevers, 1987; Kim et al., 2014). This suggests that lipases,

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