The why, when and how of lipid droplet diversity

Abdou Rachid Thiam, Mathias Beller

© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 0, 1-10 doi:10.1242/jcs.192021

COMMENTARY

The why, when and how of lipid droplet diversity

Abdou Rachid Thiam[1,*] and Mathias Beller[2,3,*]

ABSTRACT

Lipid droplets are the universal cellular organelles for the transient or long-term storage of lipids. The number, size and composition of lipid droplets vary greatly within cells in a homogenous population as well as in different cell types. The variability of intracellular lipid-storage organelles reflects the diversification of lipid droplet composition and function. Lipid droplet diversification results, for example, in two cellular lipid droplet populations that are prone to diminish and grow, respectively. The aberrant accumulation or depletion of lipids are hallmarks or causes of various human pathologies. Thus, a better understanding of the origins of lipid droplet diversification is not only a fascinating cell biology question but also potentially serves to improve comprehension of pathologies that entail the accumulation of lipids. This Commentary covers the lipid droplet life cycle and highlights the early steps during lipid droplet biogenesis, which we propose to be the potential driving forces of lipid droplet diversification.

KEY WORDS: Endoplasmic reticulum, Lipid droplets, Lipid metabolism, Organelle biogenesis, Organelle diversification, Emulsion physics

Introduction

The storage of lipids is a universal feature of cells and organisms, which evolved as a mechanism that allowed survival by buffering energy fluctuations. Within cells, lipids are stored in specialized organelles called lipid droplets. All lipid droplets share the same structure – a hydrophobic oil core of the storage lipids, which mainly comprise triacylglycerols (TAG) and sterol esters, is shielded by a phospholipid monolayer that contains specific proteins (Ohsaki et al., 2014). The lipid droplet life cycle (Fig. 1) includes lipid nucleation at the endoplasmic reticulum (ER) membrane, a growth phase that results in the potential budding and detachment of the nascent lipid droplet, followed by persistence of the lipid droplet in the cytosol before it potentially degenerates owing to the remobilization of the stored lipids by cytosolic lipases (Lass et al., 2011) or autophagy, and/or lipophagy (Cingolani and Czaja, 2016).

Throughout these steps, lipid droplets exhibit diversity. Within the past few years, several lines of evidence have demonstrated the existence of diverse multifunctional lipid droplet populations based on their distinct protein or lipid compositions (for examples, see Beller et al., 2006; Hsieh et al., 2012; Martin et al., 2005; Spandl et al., 2011; Wilfling et al., 2013). How and why lipid droplets diversify is not only an intriguing cell biology question given that lipid droplet diversity undoubtedly also has an important effect on cell and organism physiology. For example, a subset of cellular lipid droplets has been found to be prone to growth (Wilfling et al., 2013) and is thus likely to remobilize to a lesser extent. Thus, identifying lipid droplet diversification mechanisms might provide a better understanding of the pathophysiological conditions that are marked by altered lipid storage dynamics, which result, for example, in either excessive loss of lipids (Arner and Langin, 2014; Das et al., 2011; Patni and Garg, 2015; Robbins and Savage, 2015) or an increase in their storage (Byrne, 2013; Gaggini et al., 2015; Montani et al., 2004; Shulman, 2014; Sztalryd and Kimmel, 2014).

Lipid droplets are the only organelles that are surrounded by a phospholipid monolayer. As such, cytosolic lipid droplets can be considered an intracellular emulsion (Thiam et al., 2013b) whereby the hydrophobic lipid phase is dispersed in the hydrophilic cytoplasm in the form of droplets protected by a phospholipid monolayer. The monolayer that encloses lipid droplets thus has unusual properties compared to bilayer membranes, and the monolayer controls the selective binding of proteins to the lipid droplet surface (Thiam et al., 2013b). This key feature of lipid droplets has become an important point of focus in the lipid droplet field as the proteins that are bound to lipid droplets define lipid droplet identity, regulation and function. Still, how proteins selectively bind to lipid droplets remains poorly understood. Knowing the basics of emulsion physics (Thiam and Forêt, 2016; Thiam et al., 2013b) offers a unique angle to study and answer this question. Emulsion science has provided for some time a good understanding of the thermodynamic stability of droplet dispersion that is stabilized by surfactants, such as phospholipids or proteins. The use of approaches that employ emulsion science can thus be expected to have a tremendous impact on lipid droplet biology, similar to the impact of liposomal reconstitutions on membrane biology.

This Commentary summarizes the currently known examples of lipid droplet diversification and links lipid droplet diversity to the lipid droplet life cycle. Based on the available data, we discuss the potential regulatory mechanisms of protein binding to lipid droplets that allow the biogenesis of multifunctional lipid droplets (Pol et al., 2014; Welte, 2015).

Are all lipid droplets the same?

The answer is simply no. Everyone who has ever looked at the lipid droplets of different cell lines or tissues quickly realizes that lipid droplets vary within and between cell types in terms of their spatial organization, number and size (Fig. 2) (for example, see Gocze and Freeman, 1994; Herms et al., 2013; Szymanski et al., 2007). *Drosophila* S2 cells, for example, carry many small lipid droplets of equal size (Krahmer et al., 2011) (Fig. 2A). By contrast, most other cultured cells, such as monkey COS-7 kidney or murine AML12 hepatocytes (Tschapalda et al., 2016) (Fig. 2B) harbor an array of numerous and differently sized lipid droplets. The same is true for most organs *in vivo*, as is for example the case for the cells from the *Drosophila* fat storage organ, the fat body (Beller et al., 2010b). The

[1]Laboratoire de Physique Statistique, École Normale Supérieure, PSL Research University; Université Paris Diderot Sorbonne Paris-Cité; Sorbonne Universités UPMC Univ Paris 06; CNRS; 24 rue Lhomond, Paris 75005, France. [2]Institute for Mathematical Modeling of Biological Systems, Heinrich Heine University, Universitätsstr. 1, Düsseldorf 40225, Germany. [3]Systems Biology of Lipid Metabolism, Heinrich Heine University, Universitätsstr. 1, Düsseldorf 40225, Germany.

*Authors for correspondence (thiam@lps.ens.fr; mathias.beller@hhu.de)

M.B., 0000-0003-0987-0080

Journal of Cell Science (2017) 0, 1-10 doi:10.1242/jcs.192021

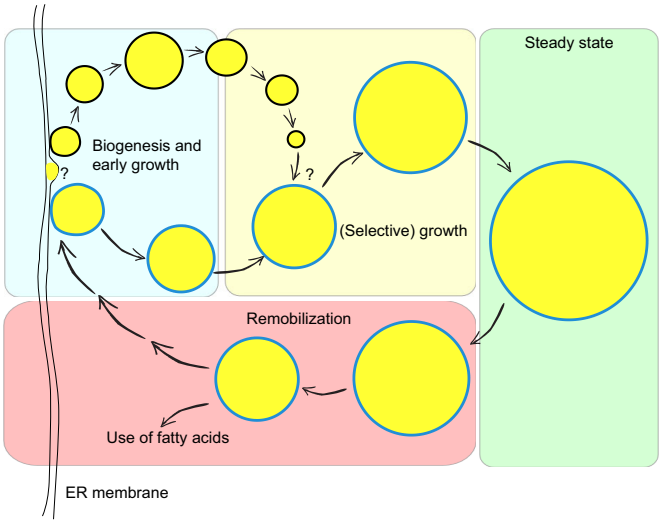

Fig. 1. The lipid droplet life cycle. Lipid droplet biogenesis and early growth is shown in the blue-shaded area. Lipid droplets arise at the endoplasmic reticulum (ER) through localized lipogenesis, which results in the accumulation of TAGs (shown in yellow) in the ER intermembrane space. Lipid accumulation culminates in the formation of a lipid lens, which grows and bends the membrane towards the cytosol. Ongoing growth results in a budding of the droplet into the cytosol. Already during the nascent state, different lipid droplet classes can arise – for example, with regard to differences in size, phospholipid or protein composition (illustrated by black- or blue-encircled lipid droplets of different sizes). The yellow-shaded area illustrates selective lipid droplet growth. Small lipid droplets disappear or transfer their lipids to larger lipid droplets through either complete fusion or transfer of only the storage lipids. The steady state is shown in the green-shaded area. Large lipid droplets persist in the cytosol for varying times depending on the cell type and physiological status of the cell. Finally, lipid droplets are remobilized (red-shaded area). Once lipid remobilization becomes activated, the droplet degenerates. Fatty acids that have been remobilized from the storage lipids then undergo esterification and re-enter the lipid droplet life cycle, are used for energy production through β-oxidation, enter anabolic reactions – such as those for membrane or hormone synthesis – or are shuttled out of the cells for use elsewhere.

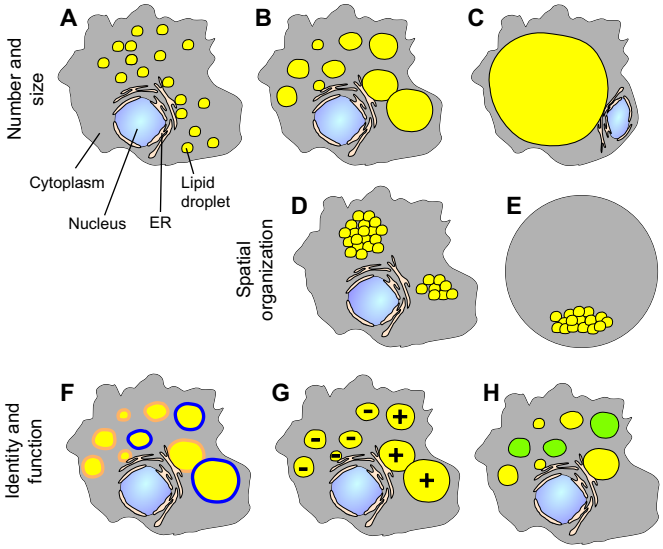

Fig. 2. Plasticity of cellular lipid storage. In cells, lipid droplets are either found to have an almost uniform size (A), show a considerable size distribution (B) or are present as giant unilocular lipid droplets in mammalian white adipocytes (C). Lipid droplets are either dispersed as shown in A,B, or clustered within cells (D). In some cell types, such as frog oocytes, lipid droplets can also be localized in a polarized manner (E). Within a single cell, the composition of individual droplets can also differ with regard to their protein coat (F), the metabolic status (G) – such as whether lipid droplets are growing (+) or shrinking (–) – or the lipid composition (H).

mammalian white adipocyte cells are probably the most extreme with regard to the way fat is stored. In these cells, a singular lipid droplet fills up the entire cytoplasm (Cushman, 1970), and even pushes the nucleus into the periphery (Fig. 2C). In addition to differences in lipid droplet size and number, the intracellular positioning of lipid droplets can also vary significantly. Cells can exhibit either dispersed (Fig. 2A), clustered (Fig. 2D) or polarized (Fig. 2E) pools of lipid droplets (Beller et al., 2010a) depending, for instance, on the cell type and metabolic state.

In addition to their appearance, the protein and lipid composition of lipid droplets can also vary between different cell types. In terms of lipids, the core of most lipid droplets is enriched with TAG and some sterol ester (Bartz et al., 2007). However, the specialized function of certain cell types is reflected by the lipid composition of their lipid droplets. Examples include the enrichment of stellate cell lipid droplets with retinyl esters (D'Ambrosio et al., 2011) and of foamy monocyte lipid droplets with the uncommon fatty acid *cis*-7-hexadecanoic acid (Guijas et al., 2016), as well as the accumulation of polyunsaturated fatty acids – such as linoleic acid in larval (Bailey et al., 2015) and peroxidated lipids – in glia cells of adult *Drosophila* (Liu et al., 2015). In terms of the lipid-droplet-associated proteins, a core set of about 50 proteins has been found to decorate lipid droplets in various cellular systems (reviewed in Hodges and Wu, 2010). However, a diverse and large number of additional proteins has been identified across many studies (a selection of studies is given in Beilstein et al., 2013; Beller et al., 2006; Cermelli et al., 2006; Chen et al., 2016; Currie et al., 2014; Dahlhoff et al., 2015; Ivashov et al., 2013; Khan et al., 2015; Khor et al., 2014; Krahmer et al., 2013; Li et al., 2016; Na et al., 2013; Schmidt et al., 2013; Su et al., 2014; Zhang et al., 2011; Zhu et al., 2015), suggesting a significant amount of variability in terms of the lipid droplet protein composition between lipid droplets isolated from different cell types.

Even within a single cell, some of the lipid droplet proteins might only associate with a subset of lipid droplets. Indeed, several examples of differential protein targeting have been revealed by fluorescence microscopy imaging of the localization of distinct proteins (Fig. 2F). For example, the annotated short chain dehydrogenase/reductase CG2254 of *Drosophila* targets lipid droplet subsets in *Drosophila* tissue culture cells and larval fat bodies (Beller et al., 2006). The triacylglycerol synthesis enzymes diacylglycerol acyltransferase 2 (DGAT2) and glycerol-phosphate acyltransferase 4 (GPAT4) (Fig. 2G) (Wilfling et al., 2013) are also found to localize to a subset of lipid droplets, which keep growing under feeding conditions and overgrow the non-decorated lipid droplets (Wilfling et al., 2013). In contrast, localization of the small GTPase Rab18 has been shown to mark lipolytic lipid droplets (Fig. 2G) (Martin et al., 2005; Pulido et al., 2011). Furthermore, different members of the founding lipid-droplet-associated protein family, the so-called perilipins (Greenberg et al., 1991; Kimmel et al., 2010), associate with distinct sets of lipid droplets. For instance, as lipid droplets increase in size during mammalian adipocyte differentiation (Wolins et al., 2006), the perilipins PLIN3 and PLIN4, PLIN2 and PLIN1 sequentially bind to them (Wolins et al., 2005). The different mammalian perilipin proteins have been suggested to have an important role in either promoting the biogenesis of lipid droplets (for example, PLIN3; Bulankina et al., 2009) or in the stabilization and regulated remobilization of lipid droplets (for example, PLIN1; Kimmel and Sztalryd, 2016). Thus,

the localization of specific perilipins provides a clue as to whether a given lipid droplet is in a transient (e.g. growing) or persistent state. The potential usefulness of perilipin localization as a diagnostic marker has been recently demonstrated in hepatosteatosis and hepatic cancerogenesis. For those diseases, the localization of PLIN1 to large lipid droplets, which are probably more difficult to remobilize, has been suggested to serve as a prognostic marker for the progression of steatosis to steatohepatitis, liver cirrhosis and cancer (Straub et al., 2008, 2010).

Differences in the lipid composition of individual lipid droplets within cells have also been described (Fig. 2H). For example, coherent anti-stokes Raman scattering (CARS) microscopy has revealed that lipid droplets within human adipose-derived stem cells and microalgae have different lipid constituents (Cavonius et al., 2015; Di Napoli et al., 2016). Furthermore, in McArh7777 rat liver cells, lipid droplets are either enriched in TAG or cholesterol esters (Hsieh et al., 2012).

The observed variation and potential discrepancies among proteomics and lipidomics studies of lipid droplets that have been isolated from various cellular systems might thus arise from any experimental and/or technical differences, or could reflect a biologically meaningful lipid droplet diversification between different cell types or tissues. To distinguish between these possibilities, lipid droplets should be purified from different cell types or tissues and processed and analyzed under identical technical, metabolic and physiological conditions. To investigate lipid droplet diversification within singular cells, the protein and lipid composition of purified lipid droplet populations should be analyzed. The ability to sort lipid droplets to high purity by using fluorescent markers and a modified fluorescence-activated cell sorting (FACS) methodology has been very elegantly demonstrated (Hsieh et al., 2012). Although this methodology has not yet been adapted extensively, it provides a powerful means to catalog lipid droplet diversification at the level of the protein and lipid composition.

Why are lipid droplets diverse?

The biological meaning of lipid droplet diversification is still unclear. In fact, there is unlikely to be a single generally valid explanation, as lipid droplets are multitasking organelles, which in addition to their energy storage function, fulfill different roles depending on the cell type and its physiological state (Pol et al., 2014; Welte, 2015). In terms of the variability of lipid droplet number in cells of a given population, it has been proposed that the lipid droplets of some cells act as 'sponges' by absorbing excess fatty acids that have been esterified into TAGs (Herms et al., 2013). Such a protective mechanism potentially also explains the accumulation of lipid droplets in glial cells during oxidative stress (Bailey et al., 2015; Liu et al., 2015). A protection from lipotoxicity could also explain the existence of small lipid droplets, which are prone to disappearing, in cells that also include growing lipid droplets (Wilfling et al., 2013). Such small transient lipid droplets could be part of a cellular 'overflow' mechanism that becomes important under conditions of elevated levels of free fatty acids, for example during the postprandial phase or in pathological scenarios.

Differences in the size and positioning of intracellular lipid droplets could help to achieve particular lipid droplet functions. The unilocular appearance of the large lipid droplet in mammalian white adipocytes probably relates to the primary function of the droplet in long-term lipid storage. Here, the reduced surface-to-volume ratio presumably helps to stabilize and protect the lipid store from improper remobilization. Upon stimulation of lipolysis, however, a number of small lipid droplets arise through the esterification of fatty acids that are liberated by the lipolytic activity. These lipid droplets could form to increase the total surface area that is accessible, for instance, to lipases to further enhance lipolytic activity (Ariotti et al., 2012) or to auxiliary proteins that were absent on the large lipid droplet, as well as possibly to mitochondria to optimize the channeling of liberated fatty acids into the β-oxidation pathway. Such a lipolysis-induced spatial repositioning of lipid droplets to mitochondria by transport along microtubules has indeed been demonstrated in Vero and COS-7 cells (Herms et al., 2015). Alternatively, the small lipid droplets could be simply formed to prevent lipotoxicity due to the sudden increase in an excess of free fatty acids. Both interpretations of the appearance of small lipid droplets during lipolysis are still under debate (Hashimoto et al., 2012; Paar et al., 2012).

When do lipid droplets diversify?

Although several pathways have been identified that are linked to the question of 'how and when do lipid droplets diversify?', the understanding of the underlying mechanisms is still lagging behind. Lipid droplet diversification can potentially arise at different steps of the lipid droplet life cycle (Fig. 1) – during lipid droplet emergence, in the steady state or over the course of remobilization. In the following, we present the different stages of the lipid droplet life cycle in detail and discuss possible steps that are crucial for lipid droplet diversification.

Lipid droplet emergence

Step 1 – lipid synthesis and nucleation

Lipid droplet biogenesis starts with the synthesis of neutral lipids by enzymes that are localized at the ER. The newly formed neutral lipids – for instance TAGs, which are the most prevalent – are transferred into the intermembrane space of the ER. When the neutral lipid concentration reaches a critical level, it becomes thermodynamically favorable that the molecules coalesce to nucleate to a lipid lens. Indeed, recent electron microscopy data clearly show the presence of such lipid lenses of around 50 nm in size (Choudhary et al., 2015). However, it is unknown whether the formation of these lenses occurs stochastically and/or on controlled sites of the ER. In fact, both mechanisms are possible and could potentially result in the biogenesis of different lipid droplets.

The nucleation of lipid lenses can be described by a phase de-mixing phenomenon, which is controlled by energetic constraints in response to the increased accumulation of TAGs in the intermembrane space of the ER (Fig. 3). Lipid nucleation thus requires the need to overcome an energy barrier. Several factors contribute to the energy barrier – the energetic costs of interactions between homo- and heterotypic molecules (i.e. interactions among TAG molecules versus interactions between TAGs and, for example, phospholipids), the thermal energy, the propensity entropy to disperse TAG molecules in the bilayer and the concentration of TAGs (Thiam and Forêt, 2016). This energy barrier might be lower in some ER sites, and these would therefore constitute the preferential sites for lipid droplet formation (Thiam and Forêt, 2016). If the energy barrier is not met, the rapid and efficient packaging of lipids into droplets will fail and, subsequently, the TAG content within the ER bilayer will increase, ultimately resulting in the formation of relatively big lipid droplets.

Defined nucleation sites with a lower energy barrier will constitute the main sites of lipid droplet formation as long as the TAG concentration in the bilayer remains below a critical

Journal of Cell Science (2017) 0, 1-10 doi:10.1242/jcs.192021

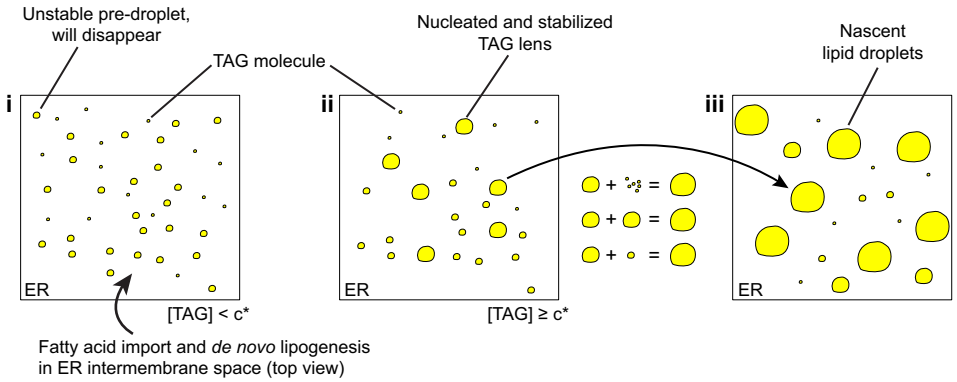

Fig. 3. Lipid nucleation within the ER membrane. The illustration represents a view from the top on the ER membrane. Lipid droplet formation requires the surpassing of a critical lipid concentration (c*) to overcome the energy barrier that prevents lipid nucleation. (i) Below the critical concentration, TAG molecules within the ER membrane are either present in a solitary state or within dynamically clustered structures (termed pre-droplets). These pre-droplets are unstable and cycle between assembly, dissociation and reassembly. (ii) Under conditions of increased TAG concentration, pre-droplets nucleate into lipid lenses at ER regions that have a low energy barrier owing to, for example, membrane perturbations or nucleating proteins (see main text and Fig. 4). (iii) During ongoing lipogenesis, lipid lenses can grow to form nascent lipid droplets. At least three possible growth mechanisms can be envisaged as illustrated here: absorption of free TAGs, fusion between different lipid lenses and pre-droplets, or transfer of lipids from disappearing to growing droplets.

concentration. This concentration is affected by the rate of TAG synthesis, the abundance and/or activity of the DGAT enzymes (Harris et al., 2011), which catalyze the final step of TAG synthesis, or the distribution of phospholipids in the membrane. At high TAG concentrations, aberrant or alternative nucleation sites could arise owing to thermal fluctuations; this could occur, for instance, in oleate-loaded cells or under pathological conditions that are marked by highly elevated levels of free fatty acids.

A nucleated lipid lens can enlarge (Fig. 3) by either acquiring more TAG generated through ongoing lipogenesis, through lateral fusion of lipid lenses that are still in the ER bilayer (Thiam and Forêt, 2016) or by a ripening process, which involves the transfer of lipids between lenses and/or nascent lipid droplets (Thiam and Forêt, 2016; Thiam et al., 2013b). This growth of the lipid lenses will result in a budding – i.e. the accumulated lipids become spherical – into a nascent lipid droplet of about 100–300 nm in size (Fig. 3). During all these steps, the droplet shares its surrounding phospholipid monolayer with the ER. Intriguingly, however, the phospholipid monolayer composition of purified lipid droplets is different from the phospholipid composition of the ER membrane (Tauchi-Sato et al., 2002; Zanghellini et al., 2010). Currently, it is unknown whether only specific phospholipids are recruited to the surface of nucleated and/or nascent lipid droplets, whether different lipid droplets are nucleated in different phospholipid environments of the ER and whether lipid droplet biogenesis occurs at sites where the ER membrane composition is altered. The concomitant occurrence of these mechanisms could be a source of lipid droplet diversity.

Nascent lipid droplets will keep growing to become mature lipid droplets – i.e. lipid droplets that reach steady state. Whether lipid droplets remain in contact with the ER at this stage, or ultimately pinch off either spontaneously or through the action of specific proteins, is not yet clear. This might also vary between different cell types and the physiological and metabolic characteristics of the given cell. Because more and more ER-derived proteins are known to target lipid droplets, it is crucial to address this question to better understand whether the identity or function of a given lipid droplet, which is tied to the composition of its proteome, is fixed or can be dynamically adjusted through the exchange of proteins between the ER and lipid droplets.

Step 2 – regulation of the early steps of lipid droplet biogenesis

The energy barrier that controls lipid droplet nucleation is very likely to be lowered at regions of the membrane where the two phospholipid monolayers of the bilayer do not adhere or are partially ‘unzipped’. Common examples of such situations are membrane bends (Fig. 4A). Here, the increased exposure of the hydrophobic interior induces stress on the membrane (Thiam and Forêt, 2016). The accumulation of TAG molecules in these defect regions will be favorable as it relieves the overall membrane stress. Nucleation sites could thus coincide with membrane bends (Fig. 4A). One way to induce similar defects is the insertion of proteins into the membrane that generate membrane curvature or hydrophobic mismatches (McMahon and Boucrot, 2015) – i.e. the total length of the hydrophobic acyl chains of the phospholipids is smaller than the bilayer thickness (Fig. 4B,C). As DGATs are the final enzymes in TAG synthesis, they are good candidates to generate lipid droplet nucleation sites through membrane bending. Most organisms, in fact, encode two DGAT isoforms (Harris et al., 2011). The isoforms possess different structural features and only show partially redundant functions (Liu et al., 2012). DGAT1 is an integral membrane protein of the ER (McFie et al., 2010), whereas membrane association of DGAT2 is likely to be caused by a hairpin structure that mediates only a partial insertion into the membrane (McFie et al., 2014; Stone et al., 2006). These different modes of membrane localization could both result in hydrophobic mismatches and membrane curvature, which can give rise to local defects that are favorable for TAG accumulation (Fig. 4B,C).

The structural differences among the DGAT proteins, as well as any potential functional differences that are not related to TAG synthesis, could not only affect lipid droplet formation but could also be the reason for the occurrence of lipid droplet subtypes (Wilfling et al., 2013). For instance, the ability of DGAT1 to laterally diffuse within the membrane is likely to be much smaller than that of DGAT2 owing to its integral membrane insertion (Fig. 4B,C). Therefore, it is possible that DGAT1-mediated TAG synthesis is locally constrained. In contrast, the spatial flexibility of DGAT2 would result in a much more disperse distribution of TAG within the ER intermembrane space. Along those lines, primary and secondary lipid droplet nucleation sites might exist. Primary lipid droplet nucleation sites would provide controlled lipid droplet biogenesis and are marked by the presence of the lipogenic

Journal of Cell Science (2017) 0, 1-10 doi:10.1242/jcs.192021

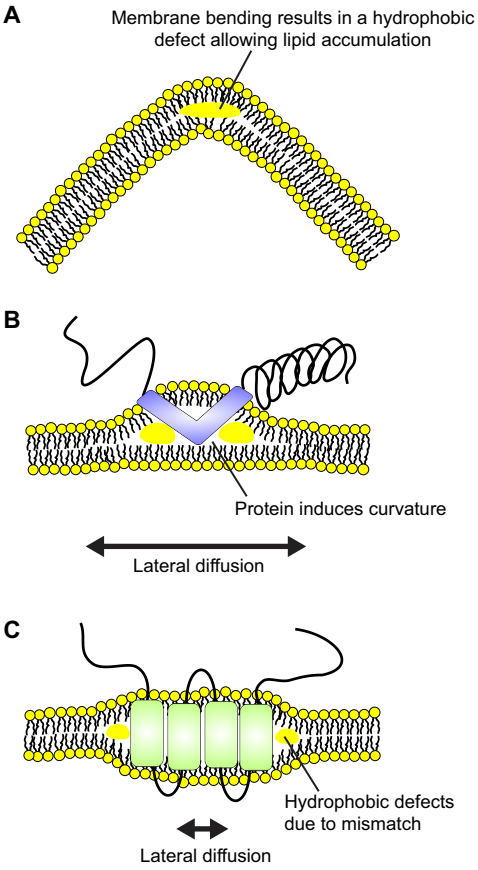

Fig. 4. Lipid accumulation requires a local perturbation of the ER membrane. Lipid droplet nucleation is facilitated by local perturbations of the ER membrane, which result, for instance, in 'unzipping' of the phospholipids. (A) Bent membranes are probable sites of lipid droplet biogenesis. The enhanced curvature results in an enlarged intermembrane space, which is potentially filled by accumulating the enzymes. (B,C) Integral or peripherally inserted proteins also promote the accumulation of lipids in the ER intermembrane space by introducing membrane defects. DGAT enzymes, which catalyze the final step of TAG synthesis, are good examples; DGAT2 (B) is peripherally inserted into the ER membrane through a hairpin structure, whereas DGAT1 is an integral ER-membrane protein (C). In addition, the different modes of membrane association potentially result in a lower (DGAT1) or higher (DGAT2) mobility of the enzymes within the ER membrane. This might result in either a locally restricted (C) or widespread distribution (B) of the synthesized TAG in the ER intermembrane space, as indicated by the different lengths of diffusions arrows.

machinery and additional proteins, which potentially facilitate the regulated biogenesis of lipid droplets and the controlled association of proteins. By contrast, secondary sites are sites of spontaneous events along the ER membrane, marked by a low nucleation energy barrier, which facilitates the appearance of lipid lenses in an unregulated manner (Thiam and Forêt, 2016). Owing to stochastic protein distribution, the lipid droplets that arise at such secondary random sites are likely to comprise a different set of proteins as compared to those lipid droplets that originate at defined primary sites. This stochastic component might also result in variations in the phospholipid or cargo lipid composition, which ultimately gives rise to different sets of lipid droplets.

Three other ER-localized protein families profoundly impact lipid droplet formation. The seipin protein (also known as FLD1 and SEI1 in yeast, and BSCL2 in mammals) appears to regulate ER organization and lipid droplet distribution, and additionally impacts lipogenesis that is coupled to lipid droplet formation (Bi et al., 2014; Cui et al., 2011; Fei et al., 2008, 2011; Wang et al., 2016; Wolinski et al., 2011). Individuals that lack functional seipin are affected by a severe form of lipodystrophy called Berardinelli–Seip congenital lipodystrophy (Magré et al., 2001). Further, the ER-localized acyl-CoA synthetase 3 (ACSL3) is rapidly recruited to sites of lipid droplet biogenesis, where it is likely to be important for stabilizing lipid droplet nucleation and lipid storage (Kassan et al., 2013). The growth of the lipid lens additionally depends on the action of the fat-storage-induced transmembrane (FIT) proteins, which are needed for the budding of lipid droplets from the ER membrane (Choudhary et al., 2015). Accordingly, a loss of FIT function in yeast and higher eukaryotes causes lipid droplets to remain in the ER membrane (Choudhary et al., 2015). Loss of function of the single FIT-encoding gene in *Caenorhabditis elegans* is lethal, suggesting that lipid droplet formation is a vital process in this organism (Choudhary et al., 2015).

The exact mechanisms by which these proteins affect lipid droplet formation sites remain to be elucidated. Nevertheless, a local lowering of the nucleation barrier and stabilization of the nucleated droplets at these sites, as recently proposed for seipin (Wang et al., 2016), could involve a remodeling of the ER membrane or a change of the lipid distribution – e.g. through a direct interaction with TAG or phospholipids.

Step 3 – protein association with nascent lipid droplets

Many proteins localize to lipid droplets through the ER (for examples, see Kory et al., 2016; Thiel et al., 2013; Zehmer et al., 2009), and it is thus likely that nascent lipid droplets recruit cytosolic or ER proteins during their emergence (Fig. 5). The site of lipid droplet nucleation, either at primary (predetermined) or secondary (random) ER sites as outlined above, can be also a determining factor of the protein coat composition of lipid droplets through multiple non-exclusive mechanisms.

With regard to lipid droplet nucleation at secondary sites, protein association is predominantly regulated by coincidental contact between a putative lipid droplet protein and the nascent droplet, as well as the overall biophysical properties. For example, association of a protein with the lipid droplet could be favored through a hydrophobic interaction with the lipid droplet oil core or the curvature of the forming lipid droplet (Fig. 5A). So far, there is only little information regarding the importance of the composition of the lipid droplet oil core for protein binding. In this context, the selective targeting of PLIN4 to lipid droplets containing cholesterol esters and of PLIN5 to those that have TAGs, could provide support for this notion (Hsieh et al., 2012). At the level of nascent lipid droplets, a number of proteins could potentially have access to a lipid droplet, but proteins with lower affinities could be displaced by competition or crowding effects (Kory et al., 2015) (Fig. 5B). This could result in the sequential formation of lipid droplets that comprise distinct pools of proteins. During a lipogenic burst with excessive lipid droplet formation on secondary sites, such a mechanism would, however, be less relevant for affinity-based lipid droplet diversification given that a hypothetically sufficient lipid droplet surface area is present to host most available proteins.

Primary ER lipid droplet nucleation sites are potentially enriched in multiple proteins, such as DGAT1, seipin, FITs and/or ACSL3 (see above). Additionally, yet to be identified, curvature-inducing proteins could locally further minimize the energy barrier that is necessary to stabilize lipid droplet nucleation. In that manner, they could control the propensity of lipid droplet formation and affect the recruitment of additional proteins to nascent droplets. This

Journal of Cell Science (2017) 0, 1-10 doi:10.1242/jcs.192021

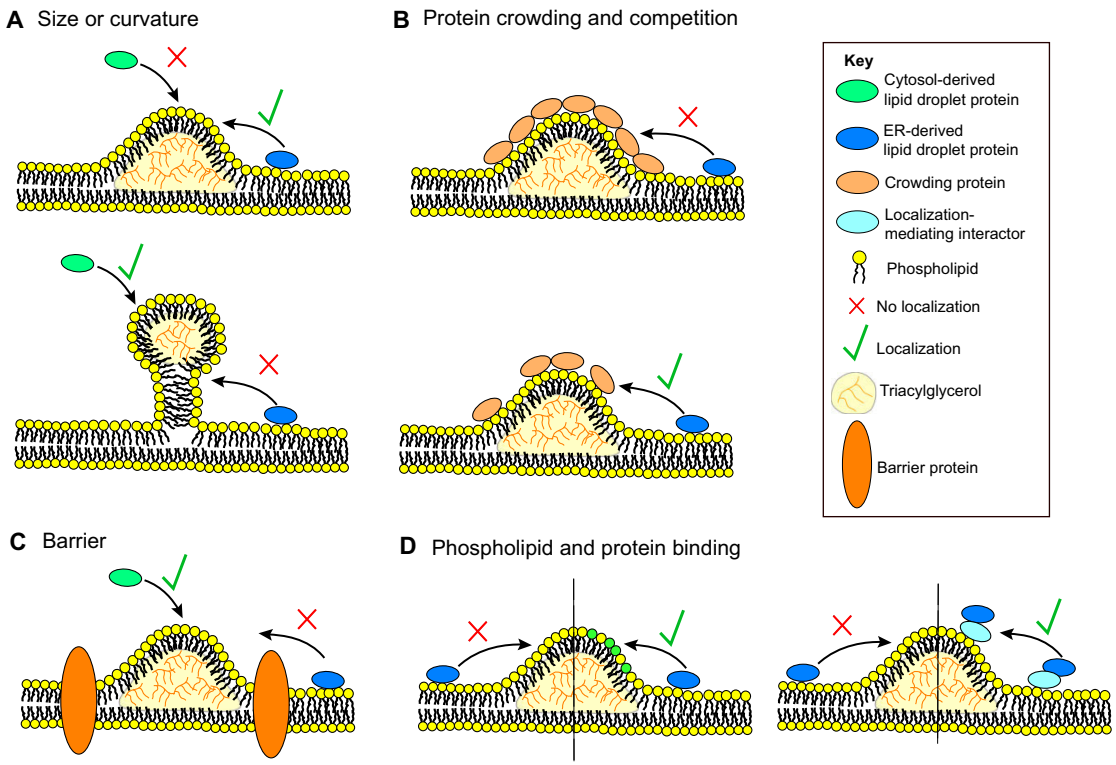

Fig. 5. Selective targeting of proteins to nascent lipid droplets. Different mutually non-exclusive mechanisms potentially control the localization of ER- or cytosol-derived proteins to nascent lipid droplets. (A) Proteins that are targeted to lipid droplets might sense certain nucleating or nascent lipid droplets through their differences in size or curvature. Some ER-derived proteins might only be able to access lipid droplets during the nascent state and lose the ability to access lipid droplets after their emergence from the ER. Cytosolic proteins, however, could still target such lipid droplets. (B) Protein crowding at the surface of nascent lipid droplets might also dictate the protein composition of the forming lipid droplet as proteins with lower binding affinity are recruited last. (C) Integral ER membrane proteins that are important for lipid droplet formation might gate the recruitment of other proteins to nascent lipid droplets. In this way, the lipid droplet surface area could be kept accessible for cytosol-derived proteins (shown), or alternatively, ER-derived proteins could be recruited to the lipid droplet surface, as has been proposed for the lipid droplet localization of the hepatitis C virus core protein through its interaction with DGAT1 (for details see main text). (D) Translocation to nascent lipid droplets could be linked to a specific phospholipid composition (left panel) or the presence of supporting proteins (right panel), which either act in concert for localization to nascent lipid droplets or are pre-loaded to the nascent lipid droplets through one of the aforementioned mechanisms.

could be achieved through the formation of a protein diffusion barrier that simply shields the lipid droplet nucleation site, or by generating membrane properties that are unfavorable for diffusion (Fig. 5C). Such a mechanism would allow the binding of cytosolic proteins to nascent lipid droplets. Indeed, several proteins target lipid droplets without having any prior membrane association, for example PLIN1 (Brasaemle et al., 1997). Finally, ER proteins present at lipid droplet biogenesis sites could transiently interact with transmembrane proteins that either promote their retention in the ER during lipid droplet formation or promote lipid droplet targeting (Fig. 5D). This mechanism, for example, is proposed to mediate the localization of the hepatitis C virus core protein to lipid droplets through interaction with the ER-localized DGAT1 enzyme (Herker et al., 2010).

Protein-induced lipid lens formation can also result in secondary lipid droplet nucleation sites. For example, damaged and/or unfolded proteins in the ER can expose hydrophobic amino acid residues to the ER intermembrane space, which results in a partial unzipping of the membrane and in ER membrane stress. This then would favor the accumulation of neutral lipid molecules and, subsequently, the formation of lipid droplets. Furthermore, these damaged proteins could be incorporated into the forming lipid droplets and subsequently degraded through microlipophagy, for example, thus representing an ER protein quality control pathway (Vevea et al., 2015).

Steady-state lipid droplets – differential protein targeting to existing lipid droplets

Several examples of the differential protein targeting to larger and cytosolic droplets exist. Among these, the above-described localization of distinct perilipin proteins to lipid droplets of increasing size during mammalian adipocyte maturation (Wolins et al., 2006) stands out. However, the basis of the binding variability of the different perilipins is currently unknown. Although PLIN3 and PLIN2 have been proposed to localize to nascent lipid droplets in the ER, the specific membrane association mechanism for PLIN4 and PLIN1 is unknown. The exchange of the ER-localized perilipins PLIN3 and PLIN2 with PLIN1 could be linked to the translation of PLIN1 on free ribosomes in combination with its degradation in the absence of lipid droplets (Servetnick et al., 1995; Xu et al., 2006). Growth of the lipid droplets – with only limited protein replenishment occurring at the same time – could result in the availability of lipid droplet surface area, which facilitates a co- or post-translational targeting of PLIN1; this, in turn, might displace already localized PLIN2 and PLIN3 owing to the higher affinity of PLIN1 for the lipid droplet phospholipid monolayer. As an alternative, the observed sequential recruitment of proteins could be based on an enzyme-mediated remodeling of the phospholipid monolayer, which would result in altered biophysical properties that are potentially recognized by the given protein. Of course, such differences in biophysical properties could also already arise during

lipid droplet nucleation owing to the particular properties of the primary or secondary nucleation sites (see above).

With regard to a secondary remodeling of the lipid droplet coat, only one mechanism has so far been described in detail. A number of independent studies have revealed a role of the coat protein complex I (COPI) machinery in the regulation of the amount of stored lipids (Beller et al., 2008; Guo et al., 2008; Soni et al., 2009). COPI is best known for its function in the retrograde trafficking of proteins and lipids from the Golgi back to the ER (Beck et al., 2009) and in the anterograde trafficking of small cargo between Golgi stacks (Pellett et al., 2013). Mechanistically, COPI activity has been shown to control the targeting of key lipid storage regulators, such as the adipose triglyceride lipase (ATGL; also known as PNPLA2) (Beller et al., 2008; Soni et al., 2009) or the lipogenic enzymes GPAT4 and DGAT2 (Wilfling et al., 2014), to the surface of lipid droplets. This is achieved through a modification of the lipid droplet surface composition by COPI-mediated budding of nano-lipid droplets from the surface of lipid droplets. *In vitro* experiments with isolated artificial lipid droplets have demonstrated that COPI alone is sufficient to bud nano-lipid droplets of 60 nm in diameter (Thiam et al., 2013a). The budding of nano-lipid droplets alters the phospholipid composition of the lipid droplet, which in turn results in an increased surface tension (Thiam et al., 2013a) that subsequently promotes the formation of bridges between cytosolic lipid droplets and the ER. Proteins and lipids can travel between the two compartments through these bridges allowing, for instance, the secondary recruitment of lipogenic enzymes to existing mature lipid droplets (Wilfling et al., 2014). Intriguingly, COPI activity is restricted to a subset of lipid droplets and, accordingly, lipogenic enzymes also only reach a subset of lipid droplets. How COPI activity is targeted to selected lipid droplets is currently unknown.

Remobilizing lipid droplets – protein targeting to lipolytic lipid droplet subsets

The small GTPase Rab18 specifically targets a subset of lipid droplets following stimulation of lipolysis (Martin et al., 2005; Pulido et al., 2011). Its localization to lipid droplets also enhances basal lipolysis rate in murine adipocytes (Pulido et al., 2011). Interestingly, the insulin-mediated activation of lipogenesis also triggers localization of Rab18 to lipid droplets in murine 3T3-L1 cells, and overexpression of Rab18 further enhances the basal lipogenesis rate (Pulido et al., 2011). Thus, Rab18 has a role in lipogenesis and lipolysis (Pulido et al., 2011). How Rab18 is targeted to subsets of lipid droplets is currently unknown. Insulin-mediated localization, however, has been found to depend on the phosphorylation of Rab18 by phosphatidylinositol-3-kinase (PI3K) (Pulido et al., 2011), suggesting post-translational modifications play an important role.

As mentioned above, targeting of the lipase ATGL to lipid droplets depends on COPI (Beller et al., 2008; Soni et al., 2009), whose activity appears to be restricted to certain subsets of lipid droplets. At the same time, COPI also mediates the targeting of lipogenic enzymes, such as GPAT4 and DGAT2, to subsets of lipid droplets (Wilfling et al., 2014). Thus, like Rab18, the COPI machinery appears to have paradoxical activities in both lipogenic and lipolytic processes. However, the proposed COPI action on lipid droplets involves multiple steps (budding of nano-lipid droplets, alteration of surface tension, formation of monolayer–bilayer bridges between the lipid droplets and the ER), which raises the possibility that other – yet to be identified – factors could mediate its switch between lipogenesis- and lipolysis-promoting effects. The same might be true for Rab18 function.

So when do lipid droplets diversify? Although lipid droplet diversification is clearly tied to the progression of the lipid droplet life cycle – in particular to the growth of lipid droplets and their secondary editing by factors such as COPI – we believe there is convincing evidence that the foundation for lipid droplet diversification, as well as the fate of lipid droplets, has already been determined during lipid droplet biogenesis. Thus, important questions to answer next are whether indeed ‘primary’ and ‘secondary’ lipid droplet biogenesis sites exist and, if so, whether they employ dedicated protein machineries that control the emergence of a lipid droplet and the associated proteins, as well as how lipid droplets are targeted by secondary editing machineries such as COPI.

Discussion and perspectives

Lipid droplet plasticity has a big impact on lipid physiology during health and disease. The emerging appreciation that not all lipid droplets are equal thus promises new insights into the regulation of lipid storage. Solving the important questions of how, when and why lipid droplets within a cell and between cells diversify, will result in a much better understanding of the regulation of the lipid droplet life cycle.

Lipid droplet diversification within a cell is probably the least characterized. So far, COPI is the only factor known to affect the localization of proteins to subsets of lipid droplets. However, COPI *a priori* only functions at already formed lipid droplets. Thus, a paramount question is whether the COPI machinery recognizes and selects certain lipid droplets to act upon stochastically or by the sensing of a particular signal. If such a specification signal exists, the next question is when and how it is imprinted. In this Commentary, we highlight the early steps of lipid droplet biogenesis at the ER as a possible time point for lipid droplet diversification and specification. The observation that the size of nascent lipid droplets can vary in response to the amount of available free fatty acids (Kassan et al., 2013) and the fact that lipid droplets can differ very early after biogenesis in terms of their protein or lipid composition support the notion that lipid droplets diversify during their emergence. As outlined above, a number of mechanisms can potentially result in the differential targeting of proteins to newly formed lipid droplets (Fig. 5), which would result in different lipid droplet protein coats. These varying protein coats would not only result in a functional diversification of lipid droplets, but such differences could also be recognized by secondary lipid droplet editing machineries such as COPI.

Another question is how stable are certain lipid droplet subsets? Although individual droplets interact with each other to exchange lipids (Gong et al., 2011), a translocation of proteins from one lipid droplet to another has not been described to the best of our knowledge. Furthermore, our own photoconversion experiments on several lipid-droplet-associated proteins do not support protein exchange between neighboring droplets (M.B., unpublished results). Still, protein translocation between droplets, as well as the heterotypic or homotypic fusion of lipid droplets (Boström et al., 2007), could affect lipid droplet diversification and needs to be studied in greater detail.

Although, the past few years have produced an ever increasing number of examples for intracellular lipid droplet diversification, we are still in the early days of understanding how lipid droplet diversification is achieved and what the functional implications of lipid droplet subsets are. Yet, the knowledge of how to enhance the prevalence of growing lipid droplets or the amount of lipid droplets that are prone to remobilization provides an entry point to identify

suitable targets to enhance the lipid content of cells for biofuel production or nutritional purposes, as well as to tackle diseases associated with lipid storage.

Acknowledgements
We thank Carole Sztalryd and the members of our groups for constructive discussions during the writing of the manuscript.

Competing interests
The authors declare no competing or financial interests.

Funding
A.R.T. is supported by grants ATIP-Avenir (from Institut National de la Santé et de la Recherche Médicale) and Programme Emergence de la Ville de Paris. M.B. is supported by grants from Bundesministerium für Bildung und Forschung (031 A 306) and Deutsche Forschungsgemeinschaft (BE 4597 / 2-1).

References

Ariotti, N., Murphy, S., Hamilton, N. A., Wu, L., Green, K., Schieber, N. L., Li, P., Martin, S. and Parton, R. G. (2012). Postlipolytic insulin-dependent remodeling of micro lipid droplets in adipocytes. *Mol. Biol. Cell* **23**, 1826-1837.
Arner, P. and Langin, D. (2014). Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance. *Trends Endocrinol. Metab.* **25**, 255-262.
Bailey, A. P., Koster, G., Guillermier, C., Hirst, E. M. A., MacRae, J. I., Lechene, C. P., Postle, A. D. and Gould, A. P. (2015). Antioxidant role for lipid droplets in a stem cell niche of Drosophila. *Cell* **163**, 340-353.
Bartz, R., Li, W.-H., Venables, B., Zehmer, J. K., Roth, M. R., Welti, R., Anderson, R. G. W., Liu, P. and Chapman, K. D. (2007). Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic. *J. Lipid Res.* **48**, 837-847.
Beck, R., Ravet, M., Wieland, F. T. and Cassel, D. (2009). The COPI system: molecular mechanisms and function. *FEBS Lett.* **583**, 2701-2709.
Beilstein, F., Bouchoux, J., Rousset, M. and Demignot, S. (2013). Proteomic analysis of lipid droplets from Caco-2/TC7 enterocytes identifies novel modulators of lipid secretion. *PLoS ONE* **8**, e53017.
Beller, M., Riedel, D., Jänsch, L., Dieterich, G., Wehland, J., Jäckle, H. and Kühnlein, R. P. (2006). Characterization of the Drosophila lipid droplet subproteome. *Mol. Cell. Proteomics* **5**, 1082-1094.
Beller, M., Sztalryd, C., Southall, N., Bell, M., Jäckle, H., Auld, D. S. and Oliver, B. (2008). COPI complex is a regulator of lipid homeostasis. *PLoS Biol.* **6**, e292.
Beller, M., Thiel, K., Thul, P. J. and Jäckle, H. (2010a). Lipid droplets: a dynamic organelle moves into focus. *FEBS Lett.* **584**, 2176-2182.
Beller, M., Bulankina, A. V., Hsiao, H.-H., Urlaub, H., Jäckle, H. and Kühnlein, R. P. (2010b). PERILIPIN-dependent control of lipid droplet structure and fat storage in Drosophila. *Cell Metab.* **12**, 521-532.
Bi, J., Wang, W., Liu, Z., Huang, X., Jiang, Q., Liu, G., Wang, Y. and Huang, X. (2014). Seipin promotes adipose tissue fat storage through the ER Ca^{2+}-ATPase SERCA. *Cell Metab.* **19**, 861-871.
Boström, P., Andersson, L., Rutberg, M., Perman, J., Lidberg, U., Johansson, B. R., Fernandez-Rodriguez, J., Ericson, J., Nilsson, T., Borén, J. et al. (2007). SNARE proteins mediate fusion between cytosolic lipid droplets and are implicated in insulin sensitivity. *Nat. Cell Biol.* **9**, 1286-1293.
Brasaemle, D. L., Barber, T., Kimmel, A. R. and Londos, C. (1997). Post-translational regulation of perilipin expression. Stabilization by stored intracellular neutral lipids. *J. Biol. Chem.* **272**, 9378-9387.
Bulankina, A. V., Deggerich, A., Wenzel, D., Mutenda, K., Wittmann, J. G., Rudolph, M. G., Burger, K. N. J. and Höning, S. (2009). TIP47 functions in the biogenesis of lipid droplets. *J. Cell Biol.* **185**, 641-655.
Byrne, C. D. (2013). Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. *Proc. Nutr. Soc.* **72**, 412-419.
Cavonius, L., Fink, H., Kiskis, J., Albers, E., Undeland, I. and Enejder, A. (2015). Imaging of lipids in microalgae with coherent anti-stokes Raman Scattering Microscopy. *Plant Physiol.* **167**, 603-616.
Cermelli, S., Guo, Y., Gross, S. P. and Welte, M. A. (2006). The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Curr. Biol.* **16**, 1783-1795.
Chen, X., Xu, S., Wei, S., Deng, Y., Li, Y., Yang, F. and Liu, P. (2016). Comparative proteomic study of fatty acid-treated myoblasts reveals role of Cox-2 in palmitate-induced insulin resistance. *Sci. Rep.* **6**, 21454.
Choudhary, V., Ojha, N., Golden, A. and Prinz, W. A. (2015). A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J. Cell Biol.* **211**, 261-271.
Cingolani, F. and Czaja, M. J. (2016). Regulation and functions of autophagic lipolysis. *Trends Endocrinol. Metab.* **27**, 696-705.
Cui, X., Wang, Y., Tang, Y., Liu, Y., Zhao, L., Deng, J., Xu, G., Peng, X., Ju, S., Liu, G. et al. (2011). Seipin ablation in mice results in severe generalized lipodystrophy. *Hum. Mol. Genet.* **20**, 3022-3030.
Currie, E., Guo, X., Christiano, R., Chitraju, C., Kory, N., Harrison, K., Haas, J., Walther, T. C. and Farese, R. V. (2014). High confidence proteomic analysis of yeast LDs identifies additional droplet proteins and reveals connections to dolichol synthesis and sterol acetylation. *J. Lipid Res.* **55**, 1465-1477.
Cushman, S. W. (1970). Structure-function relationships in the adipose cell. I. Ultrastructure of the isolated adipose cell. *J. Cell Biol.* **46**, 326-341.
Dahlhoff, M., Fröhlich, T., Arnold, G. J., Müller, U., Leonhardt, H., Zouboulis, C. C. and Schneider, M. R. (2015). Characterization of the sebocyte lipid droplet proteome reveals novel potential regulators of sebaceous lipogenesis. *Exp. Cell Res.* **332**, 146-155.
D'Ambrosio, D. N., Walewski, J. L., Clugston, R. D., Berk, P. D., Rippe, R. A. and Blaner, W. S. (2011). Distinct populations of hepatic stellate cells in the mouse liver have different capacities for retinoid and lipid storage. *PLoS ONE* **6**, e24993.
Das, S. K., Eder, S., Schauer, S., Diwoky, C., Temmel, H., Guertl, B., Gorkiewicz, G., Tamilarasan, K. P., Kumari, P., Trauner, M. et al. (2011). Adipose triglyceride lipase contributes to cancer-associated cachexia. *Science* **333**, 233-238.
Di Napoli, C., Pope, I., Masia, F., Langbein, W., Watson, P. and Borri, P. (2016). Quantitative spatiotemporal chemical profiling of individual lipid droplets by hyperspectral CARS microscopy in living human adipose-derived stem cells. *Anal. Chem.* **88**, 3677-3685.
Fei, W., Shui, G., Gaeta, B., Du, X., Kuerschner, L., Li, P., Brown, A. J., Wenk, M. R., Parton, R. G. and Yang, H. (2008). Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast. *J. Cell Biol.* **180**, 473-482.
Fei, W., Li, H., Shui, G., Kapterian, T. S., Bielby, C., Du, X., Brown, A. J., Li, P., Wenk, M. R., Liu, P. et al. (2011). Molecular characterization of seipin and its mutants: implications for seipin in triacylglycerol synthesis. *J. Lipid Res.* **52**, 2136-2147.
Gaggini, M., Saponaro, C. and Gastaldelli, A. (2015). Not all fats are created equal: adipose vs. ectopic fat, implication in cardiometabolic diseases. *Horm. Mol. Biol. Clin. Investig.* **22**, 7-18.
Gocze, P. M. and Freeman, D. A. (1994). Factors underlying the variability of lipid droplet fluorescence in MA-10 Leydig tumor cells. *Cytometry* **17**, 151-158.
Gong, J., Sun, Z., Wu, L., Xu, W., Schieber, N., Xu, D., Shui, G., Yang, H., Parton, R. G. and Li, P. (2011). Fsp27 promotes lipid droplet growth by lipid exchange and transfer at lipid droplet contact sites. *J. Cell Biol.* **195**, 953-963.
Greenberg, A. S., Egan, J. J., Wek, S. A., Garty, N. B., Blanchette-Mackie, E. J. and Londos, C. (1991). Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J. Biol. Chem.* **266**, 11341-11346.
Guijas, C., Meana, C., Astudillo, A. M., Balboa, M. A. and Balsinde, J. (2016). Foamy monocytes are enriched in cis-7-hexadecenoic fatty acid (16:1n-9), a possible biomarker for early detection of cardiovascular disease. *Cell Chem. Biol.* **23**, 689-699.
Guo, Y., Walther, T. C., Rao, M., Stuurman, N., Goshima, G., Terayama, K., Wong, J. S., Vale, R. D., Walter, P. and Farese, R. V. (2008). Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* **453**, 657-661.
Harris, C. A., Haas, J. T., Streeper, R. S., Stone, S. J., Kumari, M., Yang, K., Han, X., Brownell, N., Gross, R. W., Zechner, R. et al. (2011). DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. *J. Lipid Res.* **52**, 657-667.
Hashimoto, T., Segawa, H., Okuno, M., Kano, H., Hamaguchi, H.-O., Haraguchi, T., Hiraoka, Y., Hasui, S., Yamaguchi, T., Hirose, F. et al. (2012). Active involvement of micro-lipid droplets and lipid-droplet-associated proteins in hormone-stimulated lipolysis in adipocytes. *J. Cell Sci.* **125**, 6127-6136.
Herker, E., Harris, C., Hernandez, C., Carpentier, A., Kaehlcke, K., Rosenberg, A. R., Farese, R. V. and Ott, M. (2010). Efficient hepatitis C virus particle formation requires diacylglycerol acyltransferase-1. *Nat. Med.* **16**, 1295-1298.
Herms, A., Bosch, M., Ariotti, N., Reddy, B. J. N., Fajardo, A., Fernández-Vidal, A., Alvarez-Guaita, A., Fernández-Rojo, M. A., Rentero, C., Tebar, F. et al. (2013). Cell-to-cell heterogeneity in lipid droplets suggests a mechanism to reduce lipotoxicity. *Curr. Biol.* **23**, 1489-1496.
Herms, A., Bosch, M., Reddy, B. J. N., Schieber, N. L., Fajardo, A., Rupérez, C., Fernández-Vidal, A., Ferguson, C., Rentero, C., Tebar, F. et al. (2015). AMPK activation promotes lipid droplet dispersion on detyrosinated microtubules to increase mitochondrial fatty acid oxidation. *Nat. Commun.* **6**, 7176.
Hodges, B. D. M. and Wu, C. C. (2010). Proteomic insights into an expanded cellular role for cytoplasmic lipid droplets. *J. Lipid Res.* **51**, 262-273.
Hsieh, K., Lee, Y. K., Londos, C., Raaka, B. M., Dalen, K. T. and Kimmel, A. R. (2012). Perilipin family members preferentially sequester to either triacylglycerol-specific or cholesteryl-ester-specific intracellular lipid storage droplets. *J. Cell Sci.* **125**, 4067-4076.
Ivashov, V. A., Grillitsch, K., Koefeler, H., Leitner, E., Baeumlisberger, D., Karas, M. and Daum, G. (2013). Lipidome and proteome of lipid droplets from the methylotrophic yeast Pichia pastoris. *Biochim. Biophys. Acta* **1831**, 282-290.

Kassan, A., Herms, A., Fernández-Vidal, A., Bosch, M., Schieber, N. L., Reddy, B. J. N., Fajardo, A., Gelabert-Baldrich, M., Tebar, F., Enrich, C. et al. (2013). Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. *J. Cell Biol.* **203**, 985-1001.

Khan, S. A., Wollaston-Hayden, E. E., Markowski, T. W., Higgins, L. A. and Mashek, D. G. (2015). Quantitative analysis of the murine lipid droplet-associated proteome during diet-induced hepatic steatosis. *J. Lipid Res.* **56**, 2260-2272.

Khor, V. K., Ahrends, R., Lin, Y., Shen, W.-J., Adams, C. M., Roseman, A. N., Cortez, Y., Teruel, M. N., Azhar, S. and Kraemer, F. B. (2014). The proteome of cholesteryl-ester-enriched versus triacylglycerol-enriched lipid droplets. *PLoS ONE* **9**, e105047.

Kimmel, A. R. and Sztalryd, C. (2016). The perilipins: major cytosolic lipid droplet-associated proteins and their roles in cellular lipid storage, mobilization, and systemic homeostasis. *Annu. Rev. Nutr.* **36**, 471-509.

Kimmel, A. R., Brasaemle, D. L., McAndrews-Hill, M., Sztalryd, C. and Londos, C. (2010). Adoption of PERILIPIN as a unifying nomenclature for the mammalian PAT-family of intracellular lipid storage droplet proteins. *J. Lipid Res.* **51**, 468-471.

Kory, N., Thiam, A.-R., Farese, R. V. and Walther, T. C. (2015). Protein crowding is a determinant of lipid droplet protein composition. *Dev. Cell* **34**, 351-363.

Kory, N., Farese, R. V. and Walther, T. C. (2016). Targeting fat: mechanisms of protein localization to lipid droplets. *Trends Cell Biol.* **26**, 535-546.

Krahmer, N., Guo, Y., Wilfling, F., Hilger, M., Lingrell, S., Heger, K., Newman, H. W., Schmidt-Supprian, M., Vance, D. E., Mann, M. et al. (2011). Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidylyltransferase. *Cell Metab.* **14**, 504-515.

Krahmer, N., Hilger, M., Kory, N., Wilfling, F., Stoehr, G., Mann, M., Farese, R. V. and Walther, T. C. (2013). Protein correlation profiles identify lipid droplet proteins with high confidence. *Mol. Cell Proteomics* **12**, 1115-1126.

Lass, A., Zimmermann, R., Oberer, M. and Zechner, R. (2011). Lipolysis – a highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. *Prog. Lipid Res.* **50**, 14-27.

Li, L., Zhang, H., Wang, W., Hong, Y., Wang, J., Zhang, S., Xu, S., Shu, Q., Li, J., Yang, F. et al. (2016). Comparative proteomics reveals abnormal binding of ATGL and dysferlin on lipid droplets from pressure overload-induced dysfunctional rat hearts. *Sci. Rep.* **6**, 19782.

Liu, Q., Siloto, R. M. P., Lehner, R., Stone, S. J. and Weselake, R. J. (2012). Acyl-CoA:diacylglycerol acyltransferase: molecular biology, biochemistry and biotechnology. *Prog. Lipid Res.* **51**, 350-377.

Liu, L., Zhang, K., Sandoval, H., Yamamoto, S., Jaiswal, M., Sanz, E., Li, Z., Hui, J., Graham, B. H., Quintana, A. et al. (2015). Glial lipid droplets and ROS induced by mitochondrial defects promote neurodegeneration. *Cell* **160**, 177-190.

Magré, J., Delépine, M., Khallouf, E., Gedde-Dahl, T., Jr, Van Maldergem, L., Sobel, E., Papp, J., Meier, M., Mégarbané, A., Bachy, A. et al. (2001). Identification of the gene altered in Berardinelli–Seip congenital lipodystrophy on chromosome 11q13. *Nat. Genet.* **28**, 365-370.

Martin, S., Driessen, K., Nixon, S. J., Zerial, M. and Parton, R. G. (2005). Regulated localization of Rab18 to lipid droplets: effects of lipolytic stimulation and inhibition of lipid droplet catabolism. *J. Biol. Chem.* **280**, 42325-42335.

McFie, P. J., Stone, S. L., Banman, S. L. and Stone, S. J. (2010). Topological orientation of acyl-CoA:diacylglycerol acyltransferase-1 (DGAT1) and identification of a putative active site histidine and the role of the n terminus in dimer/tetramer formation. *J. Biol. Chem.* **285**, 37377-37387.

McFie, P. J., Jin, Y., Banman, S. L., Beauchamp, E., Berthiaume, L. G. and Stone, S. J. (2014). Characterization of the interaction of diacylglycerol acyltransferase-2 with the endoplasmic reticulum and lipid droplets. *Biochim. Biophys. Acta* **1841**, 1318-1328.

McMahon, H. and Boucrot, E. (2015). Membrane curvature at a glance. *J. Cell Sci.* **128**, 1065-1070.

Montani, J.-P., Carroll, J. F., Dwyer, T. M., Antic, V., Yang, Z. and Dulloo, A. G. (2004). Ectopic fat storage in heart, blood vessels and kidneys in the pathogenesis of cardiovascular diseases. *Int. J. Obesity* **28** Suppl. 4, S58-S65.

Na, H., Zhang, P., Ding, Y., Yang, L., Wang, Y., Zhang, H., Xie, Z., Yang, F., Cichello, S. and Liu, P. (2013). Proteomic studies of isolated lipid droplets from bacteria, C. elegans, and mammals. *Methods Cell Biol.* **116**, 1-14.

Ohsaki, Y., Suzuki, M. and Fujimoto, T. (2014). Open questions in lipid droplet biology. *Chem. Biol.* **21**, 86-96.

Paar, M., Jüngst, C., Steiner, N. A., Magnes, C., Sinner, F., Kolb, D., Lass, A., Zimmermann, R., Zumbusch, A., Kohlwein, S. D. et al. (2012). Remodeling of lipid droplets during lipolysis and growth in adipocytes. *J. Biol. Chem.* **287**, 11164-11173.

Patni, N. and Garg, A. (2015). Congenital generalized lipodystrophies–new insights into metabolic dysfunction. *Nat. Rev. Endocrinol.* **11**, 522-534.

Pellett, P. A., Dietrich, F., Bewersdorf, J., Rothman, J. E. and Lavieu, G. (2013). Inter-Golgi transport mediated by COPI-containing vesicles carrying small cargoes. *Elife* **2**, e01296.

Pol, A., Gross, S. P. and Parton, R. G. (2014). Review: biogenesis of the multifunctional lipid droplet: lipids, proteins, and sites. *J. Cell Biol.* **204**, 635-646.

Pulido, M. R., Diaz-Ruiz, A., Jiménez-Gómez, Y., Garcia-Navarro, S., Gracia-Navarro, F., Tinahones, F., López-Miranda, J., Frühbeck, G., Vázquez-Martínez, R. and Malagón, M. M. (2011). Rab18 dynamics in adipocytes in relation to lipogenesis, lipolysis and obesity. *PLoS ONE* **6**, e22931.

Robbins, A. L. and Savage, D. B. (2015). The genetics of lipid storage and human lipodystrophies. *Trends Mol. Med.* **21**, 433-438.

Schmidt, C., Ploier, B., Koch, B. and Daum, G. (2013). Analysis of yeast lipid droplet proteome and lipidome. *Methods Cell Biol.* **116**, 15-37.

Servetnick, D. A., Brasaemle, D. L., Gruia-Gray, J., Kimmel, A. R., Wolff, J. and Londos, C. (1995). Perilipins are associated with cholesteryl ester droplets in steroidogenic adrenal cortical and Leydig cells. *J. Biol. Chem.* **270**, 16970-16973.

Shulman, G. I. (2014). Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N. Engl. J. Med.* **371**, 1131-1141.

Soni, K. G., Mardones, G. A., Sougrat, R., Smirnova, E., Jackson, C. L. and Bonifacino, J. S. (2009). Coatomer-dependent protein delivery to lipid droplets. *J. Cell. Sci.* **122**, 1834-1841.

Spandl, J., Lohmann, D., Kuerschner, L., Moessinger, C. and Thiele, C. (2011). Ancient ubiquitous protein 1 (AUP1) localizes to lipid droplets and binds the E2 ubiquitin conjugase G2 (Ube2g2) via its G2 binding region. *J. Biol. Chem.* **286**, 5599-5606.

Stone, S. J., Levin, M. C. and Farese, R. V. (2006). Membrane topology and identification of key functional amino acid residues of murine acyl-CoA: diacylglycerol acyltransferase-2. *J. Biol. Chem.* **281**, 40273-40282.

Straub, B. K., Stoeffel, P., Heid, H., Zimbelmann, R. and Schirmacher, P. (2008). Differential pattern of lipid droplet-associated proteins and de novo perilipin expression in hepatocyte steatogenesis. *Hepatology* **47**, 1936-1946.

Straub, B. K., Herpel, E., Singer, S., Zimbelmann, R., Breuhahn, K., Macher-Goeppinger, S., Warth, A., Lehmann-Koch, J., Longerich, T., Heid, H. et al. (2010). Lipid droplet-associated PAT-proteins show frequent and differential expression in neoplastic steatogenesis. *Mod. Pathol.* **23**, 480-492.

Su, W., Wang, Y., Jia, X., Wu, W., Li, L., Tian, X., Li, S., Wang, C., Xu, H., Cao, J. et al. (2014). Comparative proteomic study reveals 17β-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc. Natl. Acad. Sci. USA* **111**, 11437-11442.

Sztalryd, C. and Kimmel, A. R. (2014). Perilipins: lipid droplet coat proteins adapted for tissue-specific energy storage and utilization, and lipid cytoprotection. *Biochimie* **96**, 96-101.

Szymanski, K. M., Binns, D., Bartz, R., Grishin, N. V., Li, W.-P., Agarwal, A. K., Garg, A., Anderson, R. G. W. and Goodman, J. M. (2007). The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proc. Natl. Acad. Sci. USA* **104**, 20890-20895.

Tauchi-Sato, K., Ozeki, S., Houjou, T., Taguchi, R. and Fujimoto, T. (2002). The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition. *J. Biol. Chem.* **277**, 44507-44512.

Thiam, A. R. and Forêt, L. (2016). The physics of lipid droplet nucleation, growth and budding. *Biochim. Biophys. Acta* **1861**, 715-722.

Thiam, A. R., Antonny, B., Wang, J., Delacotte, J., Wilfling, F., Walther, T. C., Beck, R., Rothman, J. E. and Pincet, F. (2013a). COPI buds 60-nm lipid droplets from reconstituted water-phospholipid-triacylglyceride interfaces, suggesting a tension clamp function. *Proc. Natl. Acad. Sci. USA* **110**, 13244-13249.

Thiam, A. R., Farese, R. V., Jr and Walther, T. C. (2013b). The biophysics and cell biology of lipid droplets. *Nat. Rev. Mol. Cell Biol.* **14**, 775-786.

Thiel, K., Heier, C., Haberl, V., Thul, P. J., Oberer, M., Lass, A., Jäckle, H. and Beller, M. (2013). The evolutionarily conserved protein CG9186 is associated with lipid droplets, required for their positioning and for fat storage. *J. Cell. Sci.* **126**, 2198-2212.

Tschapalda, K., Zhang, Y.-Q., Liu, L., Golovnina, K., Schlemper, T., Eichmann, T. O., Lal-Nag, M., Sreenivasan, U., McLenithan, J., Ziegler, S. et al. (2016). A class of Diacylglycerol Acyltransferase 1 inhibitors identified by a combination of phenotypic high-throughput screening, genomics, and genetics. *EBioMedicine* **8**, 49-59.

Vevea, J. D., Garcia, E. J., Chan, R. B., Zhou, B., Schultz, M., Paolo, G. D., McCaffery, J. M. and Pon, L. A. (2015). Role for lipid droplet biogenesis and microlipophagy in adaptation to lipid imbalance in yeast. *Dev. Cell* **35**, 584-599.

Wang, H., Becuwe, M., Housden, B. E., Chitraju, C., Porras, A. J., Graham, M. M., Liu, X. N., Thiam, A. R., Savage, D. B., Agarwal, A. K. et al. (2016). Seipin is required for converting nascent to mature lipid droplets. *Elife* **5**, e16582.

Welte, M. A. (2015). Expanding roles for lipid droplets. *Curr. Biol.* **25**, R470-R481.

Wilfling, F., Wang, H., Haas, J. T., Krahmer, N., Gould, T. J., Uchida, A., Cheng, J.-X., Graham, M., Christiano, R., Fröhlich, F. et al. (2013). Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. *Dev. Cell* **24**, 384-399.

Wilfling, F., Thiam, A. R., Olarte, M.-J., Wang, J., Beck, R., Gould, T. J., Allgeyer, E. S., Pincet, F., Bewersdorf, J., Farese, R. V. et al. (2014). Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. *Elife* **3**, e01607.

Wolins, N. E., Quaynor, B. K., Skinner, J. R., Schoenfish, M. J., Tzekov, A. and Bickel, P. E. (2005). S3-12, Adipophilin, and TIP47 package lipid in adipocytes. *J. Biol. Chem.* **280**, 19146-19155.

Wolins, N. E., Brasaemle, D. L. and Bickel, P. E. (2006). A proposed model of fat packaging by exchangeable lipid droplet proteins. *FEBS Lett.* **580**, 5484-5491.

Wolinski, H., Kolb, D., Hermann, S., Koning, R. I. and Kohlwein, S. D. (2011). A role for seipin in lipid droplet dynamics and inheritance in yeast. *J. Cell Sci.* **124**, 3894-3904.
Xu, G., Sztalryd, C. and Londos, C. (2006). Degradation of perilipin is mediated through ubiquitination-proteasome pathway. *Biochim. Biophys. Acta* **1761**, 83-90.
Zanghellini, J., Wodlei, F. and von Grünberg, H. H. (2010). Phospholipid demixing and the birth of a lipid droplet. *J. Theor. Biol.* **264**, 952-961.
Zehmer, J. K., Bartz, R., Bisel, B., Liu, P., Seemann, J. and Anderson, R. G. W. (2009). Targeting sequences of UBXD8 and AAM-B reveal that the ER has a direct role in the emergence and regression of lipid droplets. *J. Cell. Sci.* **122**, 3694-3702.
Zhang, H., Wang, Y., Li, J., Yu, J., Pu, J., Li, L., Zhang, H., Zhang, S., Peng, G., Yang, F. et al. (2011). Proteome of skeletal muscle lipid droplet reveals association with mitochondria and apolipoprotein a-I. *J. Proteome Res.* **10**, 4757-4768.
Zhu, Z., Ding, Y., Gong, Z., Yang, L., Zhang, S., Zhang, C., Lin, X., Shen, H., Zou, H., Xie, Z. et al. (2015). Dynamics of the lipid droplet proteome of the Oleaginous yeast rhodosporidium toruloides. *Eukaryot. Cell* **14**, 252-264.