



## Lipid Droplet Nucleation

Abdou Rachid Thiam, Elina Ikonen

### ► To cite this version:

Abdou Rachid Thiam, Elina Ikonen. Lipid Droplet Nucleation. Trends in Cell Biology, 2020, 31 (2), pp.108-118. 10.1016/j.tcb.2020.11.006 . hal-03046278

**HAL Id: hal-03046278**

**<https://hal.science/hal-03046278>**

Submitted on 3 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **Lipid droplet nucleation**

Abdou Rachid Thiam<sup>1</sup> and Elina Ikonen<sup>2,3</sup>

<sup>1</sup> Laboratoire de Physique de l'École Normale Supérieure, ENS, Université PSL, CNRS, Sorbonne Université, Université de Paris, F-75005 Paris, France

<sup>2</sup> Department of Anatomy and Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, 00290 Helsinki, Finland

<sup>3</sup> Minerva Foundation Institute for Medical Research, 00290 Helsinki, Finland

### **\* Correspondence to:**

Abdou Rachid Thiam  
Laboratoire de Physique Statistique,  
Ecole Normale Supérieure,  
PSL Research University,  
75005 Paris Cedex 05, France  
thiam@ens.fr

## Abstract

All living organisms can make lipid droplets (LDs), intracellular oil-in-water droplets, surrounded by a phospholipid and protein monolayer. LDs are at the nexus of cellular lipid metabolism and function in diverse biological processes. During the past decade, multidisciplinary approaches have shed light on LD assembly steps from the endoplasmic reticulum (ER): nucleation, growth, budding, and formation of a separate organelle. The molecular mechanisms underpinning these steps are, however, still elusive. This review focuses on the nucleation step, defining where and how LD assembly is initiated. We will present how membrane biophysical and physicochemical properties control this step and how proteins act on it to orchestrate LD biogenesis.

## Keywords.

Lipid droplet biogenesis, phase transitions, seipin, membrane curvature, neutral lipids, membrane chemistry

## Synopsis of lipid droplet biogenesis.

Cells respond to excess nutrients or diverse stress stimuli by fabricating neutral lipids that are subsequently packaged into LDs [1,2]. LDs, also called oil or lipid bodies [3], exert several biological functions beyond metabolism [2,4]. How neutral lipids are fabricated is rather well-known but how they are packaged into LDs remains more elusive [5–7]. Recent studies have revealed biophysical and physicochemical factors assisting in the initiation of LD assembly [8–12]. This review discusses how these factors impact LD nucleation and how proteins may modulate them to control LD assembly. We focus on the thermodynamics of phase transitions underlying LD nucleation and describe how this process is influenced by membrane proteins, lipids, and mechanical properties [13].

## Neutral lipid synthesis.

LD formation is initiated by the synthesis of **neutral lipids** (NLs) (see Glossary), which in most eukaryotes takes place in the ER bilayer [5,14] (Fig.1a). In plastids, NL synthesis may take place at the inner envelope membrane, equivalent to the plasma membrane of prokaryotes [15]. The most abundant NLs in eukaryotes are **triacylglycerols** (TGs) and **sterol esters** (SE) (Fig.1b) and the major enzymes generating them in mammals are the diacylglycerol O-acyltransferase 1 (DGAT1) and acyl-coenzyme A: cholesterol

acyltransferase 1/sterol O-acyltransferase 1 (ACAT1/SOAT1), belonging to the membrane-bound O-acyl transferase superfamily. Their cryo-electron microscopy structures show interesting similarities in the proposed catalytic mechanism [16–19]. They have an intramembrane catalytic chamber to which there are entrances from the cytoplasmic and the lateral, intramembrane side (Fig.1a). The acyl-CoA enters via the cytoplasmic tunnel and diacylglycerol (for DGAT1) or cholesterol (for ACAT1) may enter through the lateral, transmembrane side (Fig.1a). The reaction product, TG, leaves laterally, i.e. inside the bilayer, and this may also be the case for SE [16–18]. The synthesized NLs mix with phospholipids (PLs) and are diffusible in the ER bilayer [11].

These enzymes have isoforms, namely DGAT2 and ACAT2, that also catalyze the esterification reaction generating NLs, although DGAT2 is evolutionarily unrelated to DGAT1. The structures of these isoforms are not yet resolved but they may mediate the synthesis of specific NL pools under precise physiological stimulations [20–22]. ACAT2 is predominantly expressed in enterocytes and hepatocytes and esterifies dietary cholesterol [23]. DGAT2 localizes around LDs, probably mediating LD-ER contacts [24], and may synthesize and incorporate TG directly into LDs. DGAT2 is also capable of esterifying ceramides into acylceramides to be stored in LDs [25]. Hence, DGATs and ACATs may esterify other hydrocarbons with alcohol functions to generate NLs other than TG and SE.

### ***The assembly steps of lipid droplets.***

How are NLs diffusible in the ER bilayer assembled into LDs? *In vitro*, when the concentration of NLs reaches a critical level, a spontaneous condensation of NLs by phase separation can occur, leading to the formation of an oil blister within the hydrophobic bilayer region [11,26–28]: this first step of LD assembly is termed **nucleation** (Box1). Spontaneous nucleation is also likely to happen in cells.

The second step is *growth*: following nucleation principles, the oil blister has to grow, by adsorbing more NLs to become an LD or to resorb [13,29,30]. If many LDs are nucleated in parallel, it is unfavorable for all of them to grow, unless they are stabilized against **Oswald ripening** [13,31]. TGs diffuse in tubules roughly at the same rate as PLs,  $\sim 4 \mu\text{m}^2/\text{sec}$  [11], implying that a TG molecule can explore two LDs that are  $2 \mu\text{m}$  apart in a second. If LDs are not stabilized against ripening, the equilibrium state requires that only one LD, the larger one, grows at the expense of the others that resorb [13,31].

The third step consists of the *budding* of a spherical LD into the cytosol [5,14]. This step requires maintaining the PL and protein coverage of the cytosolic monolayer tighter than in

the luminal face [32]. Such asymmetry guarantees that the cytosolic leaflet remains of lower **surface tension**, promoting LD emergence in this direction [33]. If proteins bind strongly to emerging LDs from the ER lumen, LDs fail to fully emerge. This happens when lipidated apolipoprotein B100 is overexpressed in the ER lumen of hepatocytes where it keeps LDs in contact with the lumen [34].

The fourth step is likely a protein-assisted *stabilization of LD-ER contacts* or a potential *detachment* of the LD from the ER bilayer. In either case, the budded LDs remain closely tethered to the ER bilayer by several tethering proteins [35–38].

### ***Thermodynamics of lipid droplet nucleation.***

We previously detailed the thermodynamics of phase transition in the context of LD nucleation [13]. Briefly, NLs within the ER bilayer can spontaneously demix from the PLs and condense into a lens when a critical nucleation concentration (CNC) is reached [39] (Fig.2a, Figure I Box.2). The existence of a CNC is common to all phase separation phenomena [13,29]; in the context of detergents, it is the critical micellar concentration. Nucleation is associated with an energy barrier,  $E_{\text{nucleation}}$ , that needs to be overcome to generate stable NL condensates (Figure I Box.2) [1]. The CNC for triacylglycerols (TG) in a flat 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine bilayer is estimated as 3-5% (TG/PL) (Fig.2a) [26,28,40]. Below this threshold, TGs diffuse and form short-lived clusters, which size, lifetime, and appearance frequency increase with the TG concentration [1]. Beyond the CNC, some TG clusters can overcome  $E_{\text{nucleation}}$  and transit into blisters or nascent LDs (Figure I Box.2) [1–4], even though a residual fraction of TGs still mixes with the bilayer PLs [28,40,41] for entropic reasons [1]. These nucleated LDs reduce the concentration of NLs in the bilayer to below the CNC, and thereupon diminish the appearance frequency of new clusters [13,29]. In a uniform protein-free bilayer, these steps happen stochastically (Fig.2a). In cells, the compositional and structural heterogeneities of the ER membrane will induce local alterations of the energy barrier and dictate where LDs nucleate [4,13] (Fig.2b,2c).

### ***Role of membrane chemistry in lipid droplet nucleation.***

The chemical potential of NLs in a compartment represents the variation in free energy per added NL molecule. The difference in chemical potentials between the condense phase and the dilute phase quantifies how favorable it is for NLs to stay in contact with the membrane components as opposed to be into a nucleated LD [13]. The higher the chemical potential in the bilayer, the less favorable it is for NLs to remain in contact with the membrane components, and the higher the nucleation probability [13]. Therefore, the chemical potential depends on the physicochemical properties of the membrane, which implies that the CNC

and  $E_{\text{nucleation}}$  also depend on these physicochemical parameters, such as membrane proteins and PLs [13] (Figure II Box.2). Changing the membrane PL composition indeed alters the level of free TG within a bilayer [40,41] and, consequently, the nucleation efficiency of TG blisters [41]. In particular, increasing dioleoylphosphatidyl ethanolamine, cholesterol, or diacylglycerol (DAG), which are negatively curved lipids, promote the nucleation of TG blisters [8,41]. Thus, the local enrichment of these lipids in the ER may help determine LD nucleation sites. How exactly these lipids would promote LD nucleation is unclear [41] but they may alter the bilayer physical properties, such as local curvature, **surface pressure** or packing [42]. Generating PL packing defects exposes TGs to water, likely increases chemical potential and favors TG condensation [11]. In any case, these observations support the idea that local transient alterations in lipid composition can decrease the CNC and energy barrier to promote nucleation. Conversely, changes in ER PL composition could also lead to excessive accumulation of NLs in the ER membrane [8], and eventually to the nucleation of abnormally large LDs.

### ***The neutral lipid chemistry impacts nucleation.***

PLs and proteins are considered as major factors for LD assembly and NLs are often viewed as “inert” apolar molecules with no impact on LD assembly. Instead, the NL chemistry determines the interactions with the membrane constituents and sets the chemical potential. TG and SE are the most abundant NLs in eukaryotes but many others exist in specific cells from plants to animals [25,43,44] (Fig.1c-d). These diverse NLs are probably able to spontaneously condense and nucleate LDs, as shown for example for phthiocerol dimycocerosate [45]. Their contrasted chemistry proffers different chemical potentials, CNCs, and nucleation energy barriers. Differences in chemical potential are partially reflected by differential interactions with water, since the oil-water interfaces of NLs have different surface tensions and propensities to recruit proteins [46]. The relevance of the NL chemistry for LD nucleation is well illustrated by the capacity of TG to condense into droplets more efficiently than SE in a DOPC bilayer [11], or by the abnormal accumulation of TG in the ER when DG synthesis is inhibited [8]; squalene, which is a hydrocarbon and not an ester, tends to accumulate in membranes and likely has a much higher CNC, resulting in fewer and larger LDs [40,47]; likewise, retinol palmitate assembles into fewer and larger LDs than TG [48]. In short, based on several *in vitro* characterizations [11,41,46,48,49], NLs differently interact with membrane components (water, PLs, and proteins) and have different chemical potentials. This suggests that specific ER membrane settings, e.g. in PL and protein composition or curvature, may be required to trigger the condensation of a given NL into LDs. Consequently, the NL composition would determine the nucleated LD phospholipidome and proteome [4,46,48,50–55].

***Lipid droplet nucleation at tubules vs. sheets.***

The ER membrane is made of sheets and tubules whose curvature affects protein and PL sorting, coordinating biochemical reactions and vesicular trafficking [56–59]. TG and SE are also differentially sorted by membrane curvature: relative to PLs, individual TG disfavor tubules with increased curvature while SE is more enriched in them [11]. Other NLs can also be expected to show differential enrichments in curved vs. flat membranes.

Curvature generates mechanical stresses [42,60], which might be enhanced by the presence of NLs. Therefore, the prompted condensation of TG at tubules to nucleate an LD could be a means to resorb bilayer stress. Another view of this scenario is that curvature increases the chemical potential of TG. One aspect of curvature is to pull apart PLs, creating packing defects or decreasing the bilayer surface pressure, hence promoting more contacts between water and the hydrophobic bilayer core. This changes the interaction energy landscape of an NL in the curved membrane. For TG, this interaction profile likely results in an increase in chemical potential, since it preferentially moves to flatter regions or condenses [11]. In other words, the CNC and the nucleation energy barrier for TG is smaller in tubules than in flat membranes. As a result, the curvature is a catalyst for the assembly of TG LDs (Figure I-II Box.2), and the frequency of nucleated LDs would follow the abundance of ER tubules [11] (Fig.3a).

In contrast to TG, SE in tubules tends to stay as individual molecules diffusing faster than TG [11]. Increasing the SE/TG ratio decreases the nucleation efficiency at tubules [11]. SE and TG are both hydrophobic. If the spacing of PL by curvature were the sole reason for the condensation of TG, to minimize contact with water, SE would behave similarly, but it does not [11]. This suggests that other factors specifically impact SE and not TG. For example, SE could decrease tubule stress when remaining as individual molecules, as opposed to TG. Also, similarly to sterols [61], SE could alter the rigidity and surface pressure of the membrane, to a direction unfavorable for nucleation (Box.2). Finally, relative to PLs, SE and TG may have different orientations in the membrane and different interactions with membrane components. These diverse options reflect the gap in our understanding of how membrane curvature, chemistry, and mechanics impact the condensation of different NLs.

***ER membrane shaping proteins regulate nucleation.***

The role of curvature in nucleation explains, at least partly, observations made on the impact of ER shaping proteins on LD formation [10,12,62–67]. The dysfunction of several of such proteins impedes the number of formed LDs, without necessarily impacting NL levels [12].

This is the case for example for the yeast peroxisomal membrane shaping protein Pex30, or MCTP2 in mammals, [10,12] whose concomitant deletion with seipin generates large ER structures low in curvature and abnormally filled with TG [12]. This is likely because the CNC of TG (relatively to PLs) is high in these structures of zero curvature. Indeed, decreasing the PL/TG ratio by inhibiting the PC synthesis enzyme PCTY1 may enable TG to reach the CNC, and results in the recovery of LDs [12]. In this rescue experiment, fewer and larger LDs were assembled compared to normal cells, as a signature of the higher CNC in flat membranes (Fig.3a) [11]. Similarly to the PEX30/seipin deletion phenotype, when mammalian cells are subjected to hypotonic medium, they make large flat ER structures bearing high amounts of TG and are deficient in LDs [11].

Knowing the impact of membrane curvature on LD assembly, any ER shaping protein, as recently shown for atlastin, Rabs, reeps, and torsins, or other ER structuring factors, such as microtubules, septins, etc., will impact LD assembly [11,38,62–65,68,69]. The dysfunction of several of these proteins is linked to hereditary spastic paraplegia phenotypes [2,64], but whether the alteration in LD assembly is anecdotal or relevant for the disease pathogenesis, remains to be elucidated. Finally, the alteration of LD formation by ER shaping proteins might either be due to variations in the ER tubular fraction, promoting LD assembly, and/or to altered NL/PL ratio, since the synthesis of NLs, like that of PLs [70], might happen at tubules. These two contributions need to be untangled to better understand the impact of ER shaping proteins on NL levels and LD nucleation.

The inner nuclear membrane (INM) possesses metabolic activity and can synthesize TG [71,72]. This seems to be the case at least for the phospholipid: diacylglycerol acyltransferase enzyme whose yeast homolog Lro1 has its catalytic activity in the nucleus and mediates TG synthesis in the nucleus [71]. However, the generated NLs flow through the interconnections between the INM and the ER and feed cytosolic LDs [71,73]. Why these TGs do not directly assemble into nuclear LDs? An answer could be the lack of curvature at the INM. The few nuclear LDs, particularly in hepatocytes [74,75], are mostly observed at nuclear membrane invaginations [74] offering curvature. When seipin is artificially trapped from the ER to the nuclear envelope with little curvature, LD assembly happens at the seipin sites [31]. However, the careful analysis revealed that most of these LDs assembled close to ER tubules adjacent to the nuclear envelope [11].

In short, the assembly of TG LDs is catalyzed by membrane curvature as otherwise, TG simply fills membranes, incapable of undergoing efficient condensation [11]. Consequently, the relative abundance of ER tubules may serve as a proxy for projecting on the capacity of a

cell type to handle excessive TG for storage. Cells with a relatively low abundance of ER tubules may generate fewer and larger LDs due to an inefficiency in LD nucleation (Fig.3a). Such cells could also compensate for the lack of tubules and develop alternative mechanisms to facilitate LD assembly [7]. Finally, cells might dynamically shift their ER tubule/sheet ratio pending on metabolic cues, e.g. increase it to generate more LDs (Fig.3a).

### ***Role of seipin in LD nucleation.***

Seipin is an ER membrane protein capable of determining the nucleation sites of TG LDs and localizing at the ER-LD junction [31,76–80] (Fig.1c). Seipin possesses two transmembrane domains, with both N- and C-termini facing the cytoplasm and a luminal loop bridging the transmembrane domains [76]. The protein homo-oligomerizes into ring-like structures and the luminal domains of these rings [81,82], of circa 15 nm in diameter [31], resolved by cryo-electron microscopy for human, fly, and yeast, consist of 11, 12, and 10 seipin monomers, respectively [36,83,84]. The human and fly monomers possess hydrophobic helices, dipped into the bilayer, and two beta-sheets of four beta-strands each, reminiscent of the sterol-binding protein Niemann-Pick C2 [83,84]. The luminal and transmembrane domains are sufficient to rescue the LD biogenesis defect in seipin-deficient cells, indicating that they are critical for seipin function in LD formation [77].

The ER membrane curvature and physical chemistry regulate the CNC and the nucleation energy barrier (Figure I-II Box.2). The oligomeric structure of seipin may locally exacerbate topological and biochemical parameters for further decreasing the CNC or the energy barrier [13,29,85,86]. This means that (a) the probability of nucleation will be exponentially much higher at seipins than elsewhere [13] and (b) it becomes thermodynamically more favorable to drive free TGs in membranes into seipin-nucleated LDs than assembling them into new non-seipin LDs (Fig.1) [13]. Seipin is enriched at ER tubules/edges of sheets [11] and likely acts as a membrane curvature sensor or inducer [11] (Fig.3b). Induction of a local curvature by seipin could be a mechanism for inducing the nucleation of TG LDs. Swollen cells can present large onion ring-like ER structures, lacking curvature but bearing seipin [11]. Under these conditions, NLs remain in the ER membrane, and nucleation is severely impeded [11].

The seipin oligomer structure can also provide a means to locally exacerbate interactions of seipin with lipids or their synthases [9,87–90], constituting subdomains of higher nucleation probability. In particular, seipin can bind to anionic phospholipids such as PA [84] via its beta-sandwich fold, and this might locally regulate both PL and NL synthesis to provide a favorable environment for TG condensation (Fig.2c). Such an environment might prolong the contacts of TG with the oligomer and increase their probability of condensation at seipin

(Fig.2c). In simulations, the luminal domain of human seipin traps individual TGs within its ring structure in a model ER bilayer and catalyzes TG clustering at low TG concentrations, where TGs fail to cluster in the absence of seipin [85,86] (Figure I Box.2). At a given TG concentration, TG clusters preferably form inside the seipin ring (Fig. 2c). Serine residues in helices associated with the luminal membrane leaflet and delineating the inner opening of the seipin ring appear critical for TG clustering and subsequent LD formation [86].

Since curvature is sufficient to trigger the assembly of TG LDs [31,78], the enrichment of seipin at tubules would prevent erratic nucleation events triggered by the sole action of curvature [11]. If a seipin oligomer initiates an LD, it will be more favorable for NLs across a certain distance in the tubule to move to this LD (Fig.2c). Therefore, controlling all LD nucleation events requires that a critical density of seipin oligomers is maintained [31] (Fig.3b,3c). If this condition is not met, regions of seipin-deficient phenotypes will appear within the same cell (Fig.3d). This prediction is demonstrated by experiments where seipin is trapped to the nuclear envelope and LDs forming at seipins have a normal phenotype, while the rest of the ER membrane, with low seipin levels, displays a lipodystrophic phenotype [31] (Fig.3d). These observations indicate that the seipin/tubule ratio needs critical regulation to prevent uncontrolled nucleation (Fig.3c,3d).

It is more favorable for TGs to move to seipin-nucleated LDs than to assemble into new seipin-negative ones [13]. This is because seipin-LDs have already overcome the nucleation barrier. However, because LDs can be heterogeneous in size, moving TG toward larger LD would be more favorable because of their lower internal **Laplace pressure** [13,91]. To ensure that all nucleated LDs grow, NLs need to be directed towards the smaller LDs, combating the Laplace pressure [31]. Indeed, if seipin is removed from LD-ER junctions, smaller LDs lose TG for larger LDs through the ER bilayer [31]. Finally, given the diversity of NLs, it may be that seipin mainly functions in TG LD biogenesis, but not universally for all NLs [48].

### ***Role of seipin co-workers in LD nucleation.***

Recent works indicate that seipin co-operates with and forms an LD assembly complex with promethin/LDAF1 (yeast Ldo 16/45) [78,92–94]. Promethin also forms distinct domains in the ER and several of these foci associate with seipin [78]. Seipin complexes synthesized under lipid-poor conditions do not readily associate with promethin but when NL synthesis is initiated, the fraction of seipins associating with promethin increases [86]. This suggests that NLs facilitate the formation of seipin-promethin complexes. LDs form at sites typically occupied by both seipin and promethin, and upon subsequent LD growth, promethin

relocates to the LD surface thanks to its hairpin topology [92], while seipin remains at the ER-LD junction [78].

Additional players defining the nature of the LD forming ER subdomain, and cooperating with seipin in LD formation, are also involved[95]. In yeast, seipin (Fld1) and nuclear envelope morphology protein 1 (Nem1) localize to the ER, independently of each other, but both mark LD nucleation sites and co-localize to ER-LD contact sites [9]. Nem1 is an activator of the DAG synthesizing Pah1 (mammalian lipin) enzyme. Upon activation of TG synthesis, the association of Fld1 and Nem1 increases, and this recruits the TG synthase Lro1, spatially coupling TG formation and storage sites. Importantly, a catalytically inactive Lro1 does not associate with the Fld1-Nem1 complex, implying that TG is needed for this association. Both Fld1 and Nem1 are required for the subdomains to recruit additional LD biogenesis factors, such as yeast homolog of FIT2 (Yft2), Pex30, and perilipin1 [9].

### **Concluding Remarks**

The fundamental physicochemical concepts that underlie the phenomenon of LD nucleation are based on classical principles of thermodynamics and mechanics. When these are combined with experimental findings from *in vitro* model membranes, *in vivo* cell membranes, and *in silico* computational approaches, a comprehensive understanding of this process is starting to emerge. At present, several outstanding questions remain to be addressed, see Outstanding Questions Box. To start with, most LDs are not constituted by a pure, single NL species but by mixtures of them [43]. We understand little how the complex NL chemistry impacts LD nucleation and if LDs of heterogeneous composition are nucleated from a continuous ER network [4,11]. *In vitro*, it is not possible to generate pure SE LDs but in cells, predominantly SE containing NLs can be formed in the presence of excess cholesterol when TG synthesizing enzymes are inhibited. What solubilizes the SE to enable the nucleation of such LDs, is not known.

Furthermore, is the role of seipin dedicated to TG, and perhaps SE, LD nucleation? What are the specific functions of seipin partners in LD nucleation and how are the LD assembly protein complexes spatiotemporally assembled in different systems? In this context, we also need to understand how ER organization beyond tubules and sheets, such as 3-way junctions, domains involved in protein translocation and folding, etc., impact NL distribution and LD assembly complexes. This, in turn, is related to more physiological questions of LD nucleation mechanisms in different cell types and metabolic conditions: the process in hepatocytes or enterocytes may differ considerably from that in adipocytes or white blood cells. What is foreseeable is that LD nucleation will keep inciting an increasing array of

investigators, from biofuels to food production and health care, spurred by megatrends such as pandemic obesity and increasing energy demand.

## **Acknowledgements**

We thank our team members and Dr. Lionel Fôret for critical discussions and advice. This study was supported by the Academy of Finland (grants 282192, 312491, 307415 to E.I.), Sigrid Juselius Foundation (E.I.), and ANR-17-CE11-0003-NANODROP and ANR-18-CE11-0012-01-MOBIL to A.R.T. We apologize to our colleagues whose works could not be cited due to space restrictions.

## 372 References

- 373 1 Jarc, E. and Petan, T. (2020) A twist of FATE: Lipid droplets and inflammatory lipid  
374 mediators. *Biochimie* 169, 69–87
- 375 2 Welte, M.A. and Gould, A.P. (2017) Lipid droplet functions beyond energy storage.  
376 *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862, 1260–1272
- 377 3 Murphy, D.J. (2001) The biogenesis and functions of lipid bodies in animals, plants and  
378 microorganisms. *Prog. Lipid Res.* 40, 325–438
- 379 4 Thiam, A.R. and Beller, M. (2017) The why, when and how of lipid droplet diversity. *J Cell*  
380 *Sci* 130, 315–324
- 381 5 Walther, T.C. *et al.* (2017) Lipid droplet biogenesis. *Annu. Rev. Cell Dev. Biol.* 33, 491–  
382 510
- 383 6 Nettebrock, N.T. and Bohnert, M. (2019) Born this way–Biogenesis of lipid droplets from  
384 specialized ER subdomains. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids*
- 385 7 Chapman, K.D. *et al.* (2019) Mechanisms of lipid droplet biogenesis. *Biochem. J.* 476,  
386 1929–1942
- 387 8 Adeyo, O. *et al.* (2011) The yeast lipin orthologue Pah1p is important for biogenesis of  
388 lipid droplets. *J. Cell Biol.* 192, 1043–1055
- 389 9 Choudhary, V. *et al.* (2020) Seipin and Nem1 establish discrete ER subdomains to  
390 initiate yeast lipid droplet biogenesis. *J. Cell Biol.* 219,
- 391 10 Joshi, A.S. *et al.* (2018) Lipid droplet and peroxisome biogenesis occur at the same ER  
392 subdomains. *Nat. Commun.* 9, 2940
- 393 11 Santinho, A. *et al.* (2020) Membrane Curvature Catalyzes Lipid Droplet Assembly. *Curr.*  
394 *Biol.* 30, 2481-2494.e6
- 395 12 Wang, S. *et al.* (2018) Seipin and the membrane-shaping protein Pex30 cooperate in  
396 organelle budding from the endoplasmic reticulum. *Nat. Commun.* 9, 2939
- 397 13 Thiam, A.R. and Forêt, L. (2016) The physics of lipid droplet nucleation, growth and  
398 budding. *Biochim. Biophys. Acta* 1861, 715–722
- 399 14 Olzmann, J.A. and Carvalho, P. (2018) Dynamics and functions of lipid droplets. *Nat.*  
400 *Rev. Mol. Cell Biol.*
- 401 15 Murphy, D.J. and Vance, J. (1999) Mechanisms of lipid-body formation. *Trends Biochem.*  
402 *Sci.* 24, 109–115
- 403 16 Qian, H. *et al.* (2020) Structural basis for catalysis and substrate specificity of human  
404 ACAT1. *Nature*
- 405 17 Sui, X. *et al.* (2020) Structure and catalytic mechanism of a human triacylglycerol-  
406 synthesis enzyme. *Nature*
- 407 18 Guan, C. *et al.* (2020) Structural insights into the inhibition mechanism of human sterol  
408 O-acyltransferase 1 by a competitive inhibitor. *Nat. Commun.* 11, 1–11
- 409 19 Ma, D. *et al.* (2018) Crystal structure of a membrane-bound O-acyltransferase. *Nature*  
410 562, 286–290
- 411 20 Nguyen, T.B. *et al.* (2017) DGAT1-dependent lipid droplet biogenesis protects  
412 mitochondrial function during starvation-induced autophagy. *Dev. Cell* 42, 9–21
- 413 21 Chitraju, C. *et al.* (2017) Triglyceride synthesis by DGAT1 protects adipocytes from lipid-  
414 induced ER stress during lipolysis. *Cell Metab.* 26, 407–418
- 415 22 Hung, Y.-H. *et al.* (2017) Dgat1 and Dgat2 regulate enterocyte triacylglycerol distribution  
416 and alter proteins associated with cytoplasmic lipid droplets in response to dietary fat.  
417 *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* 1862, 600–614
- 418 23 Chang, C.C. *et al.* (2000) Immunological quantitation and localization of ACAT-1 and  
419 ACAT-2 in human liver and small intestine. *J. Biol. Chem.* 275, 28083–28092
- 420 24 McFie, P.J. *et al.* (2018) Diacylglycerol acyltransferase-2 contains a c-terminal sequence  
421 that interacts with lipid droplets. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* 1863,  
422 1068–1081
- 423 25 Senkal, C.E. *et al.* (2017) Ceramide is metabolized to acylceramide and stored in lipid  
424 droplets. *Cell Metab.* 25, 686–697

- 26 Hamilton, J.A. (1989) Interactions of triglycerides with phospholipids: incorporation into the bilayer structure and formation of emulsions. *Biochemistry* 28, 2514–2520
- 27 Kassan, A. *et al.* (2013) Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. *J Cell Biol* 203, 985–1001
- 28 Khandelia, H. *et al.* (2010) Triglyceride blisters in lipid bilayers: implications for lipid droplet biogenesis and the mobile lipid signal in cancer cell membranes. *PLoS One* 5, e12811
- 29 Karthika, S. *et al.* (2016) A review of classical and nonclassical nucleation theories. *Cryst. Growth Des.* 16, 6663–6681
- 30 Kelton, K. and Greer, A.L. (2010) *Nucleation in condensed matter: applications in materials and biology*, 15Elsevier.
- 31 Salo, V.T. *et al.* (2019) Seipin facilitates triglyceride flow to lipid droplet and counteracts droplet ripening via endoplasmic reticulum contact. *Dev. Cell* 50, 478–493
- 32 Chorlay, A. *et al.* (2019) Membrane Asymmetry Imposes Directionality on Lipid Droplet Emergence from the ER. *Dev. Cell* 0,
- 33 Chorlay, A. and Thiam, A.R. (2018) An asymmetry in monolayer tension regulates lipid droplet budding direction. *Biophys. J.* 114, 631–640
- 34 Ohsaki, Y. *et al.* (2008) Lipid droplets are arrested in the ER membrane by tight binding of lipidated apolipoprotein B-100. *J. Cell Sci.* 121, 2415–2422
- 35 Bohnert, M. (2020) Tethering Fat: Tethers in Lipid Droplet Contact Sites. *Contact* 3, 2515256420908142
- 36 Henne, M. *et al.* (2019) Spatial compartmentalization of lipid droplet biogenesis. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids*
- 37 Salo, V.T. and Ikonen, E. (2019) Moving out but keeping in touch: contacts between endoplasmic reticulum and lipid droplets. *Curr. Opin. Cell Biol.* 57, 64–70
- 38 Xu, D. *et al.* (2018) Rab18 promotes lipid droplet (LD) growth by tethering the ER to LDs through SNARE and NRZ interactions. *J Cell Biol* 217, 975–995
- 39 Choudhary, V. *et al.* (2015) A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J Cell Biol* 211, 261–271
- 40 Ben M'barek, K. *et al.* (2017) ER Membrane Phospholipids and Surface Tension Control Cellular Lipid Droplet Formation. *Dev. Cell* 41, 591-604.e7
- 41 Zoni, V. *et al.* (2020) Lipid droplet biogenesis is driven by liquid-liquid phase separation. *Dev.-CELL--20-00075*
- 42 Marsh, D. (2007) Lateral Pressure Profile, Spontaneous Curvature Frustration, and the Incorporation and Conformation of Proteins in Membranes. *Biophys. J.* 93, 3884–3899
- 43 van Wijk, K.J. and Kessler, F. (2017) Plastoglobuli: plastid microcompartments with integrated functions in metabolism, plastid developmental transitions, and environmental adaptation. *Annu. Rev. Plant Biol.* 68, 253–289
- 44 Ajat, M. *et al.* (2017) Hepatic stellate cells retain the capacity to synthesize retinyl esters and to store neutral lipids in small lipid droplets in the absence of LRAT. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* 1862, 176–187
- 45 Augenstreich, J. *et al.* (2019) The conical shape of DIM lipids promotes Mycobacterium tuberculosis infection of macrophages. *Proc. Natl. Acad. Sci.* 116, 25649–25658
- 46 Chorlay, A. and Thiam, A.R. (2020) Neutral lipids regulate amphipathic helix affinity for model lipid droplets. *J. Cell Biol.* 219,
- 47 Spanova, M. *et al.* (2012) Influence of squalene on lipid particle/droplet and membrane organization in the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* 1821, 647–653
- 48 Molenaar, M.R. *et al.* (2019) Lecithin: Retinol Acyl Transferase (LRAT) induces the formation of lipid droplets. *bioRxiv*
- 49 Caillon, L. *et al.* (2020) Triacylglycerols sequester monotopic membrane proteins to lipid droplets. *Nat. Commun.* 11, 1–12
- 50 Hsieh, K. *et al.* (2012) Perilipin family members preferentially sequester to either triacylglycerol-specific or cholesteryl-ester-specific intracellular lipid storage droplets. *J Cell Sci* 125, 4067–4076

- 51 Davidi, L. *et al.* (2015) Proteome analysis of cytoplasmatic and plastidic  $\beta$ -carotene lipid droplets in *Dunaliella bardawil*. *Plant Physiol.* 167, 60–79
- 52 Fei, W. *et al.* (2011) The size and phospholipid composition of lipid droplets can influence their proteome. *Biochem. Biophys. Res. Commun.* 415, 455–462
- 53 Meyers, A. *et al.* (2017) The protein and neutral lipid composition of lipid droplets isolated from the fission yeast, *Schizosaccharomyces pombe*. *J. Microbiol.* 55, 112–122
- 54 Meyers, A. *et al.* (2016) Lipid droplets form from distinct regions of the cell in the fission yeast *Schizosaccharomyces pombe*. *Traffic* 17, 657–669
- 55 Vrablik, T.L. *et al.* (2015) Lipidomic and proteomic analysis of *Caenorhabditis elegans* lipid droplets and identification of ACS-4 as a lipid droplet-associated protein. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* 1851, 1337–1345
- 56 Westrate, L.M. *et al.* (2015) Form follows function: the importance of endoplasmic reticulum shape. *Annu. Rev. Biochem.* 84, 791–811
- 57 Voeltz, G.K. and Prinz, W.A. (2007) Sheets, ribbons and tubules—how organelles get their shape. *Nat. Rev. Mol. Cell Biol.* 8, 258–264
- 58 Lagace, T.A. and Ridgway, N.D. (2013) The role of phospholipids in the biological activity and structure of the endoplasmic reticulum. *Biochim. Biophys. Acta BBA-Mol. Cell Res.* 1833, 2499–2510
- 59 Tian, A. and Baumgart, T. (2009) Sorting of lipids and proteins in membrane curvature gradients. *Biophys. J.* 96, 2676–2688
- 60 Hossein, A. and Deserno, M. (2020) Spontaneous curvature, differential stress, and bending modulus of asymmetric lipid membranes. *Biophys. J.* 118, 624–642
- 61 Dimova, R. (2014) Recent developments in the field of bending rigidity measurements on membranes. *Adv. Colloid Interface Sci.* 208, 225–234
- 62 Klemm, R.W. *et al.* (2013) A conserved role for atlastin GTPases in regulating lipid droplet size. *Cell Rep.* 3, 1465–1475
- 63 Falk, J. *et al.* (2014) Functional Mutation Analysis Provides Evidence for a Role of REEP 1 in Lipid Droplet Biology. *Hum. Mutat.* 35, 497–504
- 64 Renvoisé, B. *et al.* (2016) Reep1 null mice reveal a converging role for hereditary spastic paraplegia proteins in lipid droplet regulation. *Hum. Mol. Genet.* 25, 5111–5125
- 65 Boström, P. *et al.* (2005) Cytosolic lipid droplets increase in size by microtubule-dependent complex formation. *Arterioscler. Thromb. Vasc. Biol.* 25, 1945–1951
- 66 Joshi, A.S. *et al.* (2017) Organelle biogenesis in the endoplasmic reticulum. *Nat. Cell Biol.* 19, 876
- 67 Hariri, H. *et al.* (2018) Lipid droplet biogenesis is spatially coordinated at ER–vacuole contacts under nutritional stress. *EMBO Rep.* 19, 57–72
- 68 Akil, A. *et al.* (2016) Septin 9 induces lipid droplets growth by a phosphatidylinositol-5-phosphate and microtubule-dependent mechanism hijacked by HCV. *Nat. Commun.* 7, 1–19
- 69 Grillet, M. *et al.* (2016) Torsins are essential regulators of cellular lipid metabolism. *Dev. Cell* 38, 235–247
- 70 Schwarz, D.S. and Blower, M.D. (2016) The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell. Mol. Life Sci.* 73, 79–94
- 71 Barbosa, A.D. *et al.* (2019) Compartmentalized synthesis of triacylglycerol at the inner nuclear membrane regulates nuclear organization. *Dev. Cell* 50, 755–766
- 72 Romanauska, A. and Köhler, A. (2018) The inner nuclear membrane is a metabolically active territory that generates nuclear lipid droplets. *Cell* 174, 700–715
- 73 Renne, M.F. *et al.* (2020) , Lipid droplet biogenesis: A mystery “unmixing”? , in *Seminars in Cell & Developmental Biology*
- 74 Ohsaki, Y. *et al.* (2016) PML isoform II plays a critical role in nuclear lipid droplet formation. *J. Cell Biol.* 212, 29–38
- 75 Layerenza, J.P. *et al.* (2013) Nuclear lipid droplets: a novel nuclear domain. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* 1831, 327–340
- 76 Bohnert, M. (2018) Wrapping up the fats—a structure of the lipid droplet biogenesis protein seipin. *J Cell Biol* 217, 4053–4054

- 77 Cartwright, B.R. *et al.* (2015) Seipin performs dissectible functions in promoting lipid droplet biogenesis and regulating droplet morphology. *Mol. Biol. Cell* 26, 726–739
- 78 Chung, J. *et al.* (2019) LDAF1 and Seipin Form a Lipid Droplet Assembly Complex. *Dev. Cell*
- 79 Salo, V.T. *et al.* (2016) Seipin regulates ER–lipid droplet contacts and cargo delivery. *EMBO J.* 35, 2699–2716
- 80 Szymanski, K.M. *et al.* (2007) The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proc. Natl. Acad. Sci.* 104, 20890–20895
- 81 Binns, D. *et al.* (2010) Seipin is a discrete homooligomer. *Biochemistry* 49, 10747–10755
- 82 Lundin, C. *et al.* (2006) Membrane topology of the human seipin protein. *FEBS Lett.* 580, 2281–2284
- 83 Sui, X. *et al.* (2018) Cryo–electron microscopy structure of the lipid droplet–formation protein seipin. *J Cell Biol* 217, 4080–4091
- 84 Yan, R. *et al.* (2018) Human SEIPIN binds anionic phospholipids. *Dev. Cell* 47, 248–256
- 85 Zoni, V. *et al.* (2020) Seipin accumulates and traps diacylglycerols and triglycerides in its ring-like structure. *bioRxiv*
- 86 Prasanna, X. *et al.* (2020) Seipin traps triacylglycerols to facilitate their nanoscale clustering in the ER membrane. *bioRxiv* DOI: 10.1101/2020.10.26.355065
- 87 Pagac, M. *et al.* (2016) SEIPIN regulates lipid droplet expansion and adipocyte development by modulating the activity of glycerol-3-phosphate acyltransferase. *Cell Rep.* 17, 1546–1559
- 88 Boutet, E. *et al.* (2009) Seipin deficiency alters fatty acid  $\Delta 9$  desaturation and lipid droplet formation in Berardinelli-Seip congenital lipodystrophy. *Biochimie* 91, 796–803
- 89 Sim, M.M. *et al.* (2013) The human lipodystrophy protein seipin is an ER membrane adaptor for the adipogenic PA phosphatase lipin 1. *Mol. Metab.* 2, 38–46
- 90 Talukder, M.M.U. *et al.* (2015) Seipin oligomers can interact directly with AGPAT2 and lipin 1, physically scaffolding critical regulators of adipogenesis. *Mol. Metab.* 4, 199–209
- 91 Thiam, A.R. *et al.* (2013) The biophysics and cell biology of lipid droplets. *Nat. Rev. Mol. Cell Biol.* 14, 775
- 92 Castro, I.G. *et al.* (2019) Promethin Is a Conserved Seipin Partner Protein. *Cells* 8, 268
- 93 Teixeira, V. *et al.* (2018) Regulation of lipid droplets by metabolically controlled Ldo isoforms. *J Cell Biol* 217, 127–138
- 94 Eisenberg-Bord, M. *et al.* (2018) Identification of seipin-linked factors that act as determinants of a lipid droplet subpopulation. *J Cell Biol* 217, 269–282
- 95 Greer, M.S. *et al.* (2020) SEIPIN isoforms interact with the membrane-tethering protein VAP27-1 for lipid droplet formation. *Plant Cell* 32, 2932–2950
- 96 Sagui, C. and Grant, M. (1999) Theory of nucleation and growth during phase separation. *Phys. Rev. E* 59, 4175
- 97 Hyman, A.A. *et al.* (2014) Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* 30, 39–58
- 98 Rothman, J.E. (2019) Jim’s View: Is the Golgi stack a phase-separated liquid crystal? *FEBS Lett.* 593, 2701–2705
- 99 Dignon, G.L. *et al.* (2020) Biomolecular Phase Separation: From Molecular Driving Forces to Macroscopic Properties. *Annu. Rev. Phys. Chem.* 71, 53–75

## Glossary

**Neutral lipids:** hydrophobic apolar molecules, lacking charged groups. Usually optimal forms of energy storage rich in carbon. Can differ between cell types and metabolic states.

**Triacylglycerols:** three fatty acids esterified to each of the three alcohol groups of glycerol. Most abundant neutral lipids in eukaryotes that serve for energy and membrane lipid storage. Often referred to as triglycerides. Mostly stored in adipocytes.

**Sterol ester:** a fatty acid esterified to the alcohol of a sterol. The second most abundant neutral lipid after triglycerides. Ubiquitous in yeast. In mammals, mostly present in macrophages, adrenals and blood low-density lipoproteins as the storage form of cholesterol.

**Surface tension:** energy per area cost for exposing two fluids of different nature. Acts to minimize the interface, which is why droplets are spherical. Oil/water surface tension is decreased by the adsorption of amphiphilic molecules, such as phospholipids or amphipathic proteins, masking the interface. Surface tension of a bilayer is close to zero because of their similar inner and outer aqueous phases. For both monolayers and bilayers, the tighter the phospholipid packing, the lower the surface tension.

**Surface pressure:** the lateral pressure acting on a monolayer or a bilayer. Considers the different interactions at the interface, such as those between phospholipids, phospholipids, and neutral lipids, or phospholipids/neutral lipids and water. Lateral pressure is lower when phospholipids are pulled apart or when surface tension is augmented.

**Laplace pressure:** the pressure difference across a curved interface. For LDs, proportional to the surface tension of the interface divided by the droplets' curvature. Large droplets have a lower internal pressure than smaller ones, for a given surface tension. Compared with droplets, the Laplace pressure of bilayers is almost zero, because of the low tension.

**Ostwald ripening:** leakage of materials due to chemical potential differences. Small particles/droplets of large internal pressure spontaneously lose their molecules for bigger particles/droplets of lower internal pressure, through a continuous medium where the molecules are soluble. Ripening leads to LD heterogeneity and the eventual disappearance of small LDs.

**Box1. Basic principles of nucleation phenomena.**

Nucleation is a century-old thermodynamics principle, thoroughly explored theoretically and experimentally in diverse contexts [30,96]. Nucleation is the onset of phase separation or unmixing of constituents of a blend, e.g. of liquids, gases, or solids. It happens when it becomes energetically favorable to decrease the contact between foreign molecules within the mixture and it can be triggered by changing a physical chemistry parameter of the mixture. Examples of nucleation phenomena are the onset of salt crystallization upon reducing water concentration, or the condensation of water molecules from a gas state into raindrops, catalyzed by/at dust present in the air. In biology, nucleation emerged during the past decade as a crucial step of phase separating phenomena that govern the homeostasis of organelles [97]. It is involved in the assembly of phospholipid bilayer-bounded organelles, such as the Golgi apparatus [98], but also in the biogenesis of monolayer-encircled organelles, such as LDs [13] or membraneless organelles devoid of phospholipids called condensates [99]. How and where organelle nucleation is triggered has recently become a central question in biology [99].

**Box2. The nucleation energy barrier.**

An energy barrier needs to be overcome for nucleation to occur. The lower this energy barrier, the higher is the probability to nucleate (Figure I). The height of the energy arises from the competition between antagonist contributions involving chemical potentials and interfacial energies [13].

**Chemical potentials.** Molecules constituting a blend tend to demix because mixing generally costs energy. For demixing, the interaction of an NL with the bilayer components has to cost more energy than the interaction between NLs: the chemical potential of an NL within a PL bilayer,  $\mu(\text{NL})_{\text{bilayer}}$ , needs to be larger than that within LDs,  $\mu(\text{NL})_{\text{LD}}$ . If this condition is unmet, entropy is sufficient to keep NLs mixing with the membrane constituents, thereby hampering nucleation. The chemical potential of NLs in membranes  $\mu(\text{NL})_{\text{bilayer}}$  is likely altered as soon as features of the membrane are varied, e.g. the membrane biophysical and physicochemical factors, protein density, and composition, etc. (Figure II). Increasing  $\mu(\text{NL})_{\text{bilayer}}$  makes the NL-membrane mixing more unfavorable, which decreases the energy barrier and promotes nucleation (Fig. 2a). The gap in chemical potentials modulates the height of the energy barrier.

**LD interfacial energies.** Membrane mechanics has several inputs into the energy barrier that can work for or against nucleation. There is for instance an energy penalty coming from the bilayer leaflet deformation during nucleation; for nucleation to occur, the bilayer leaflets have to be unzipped and its monolayers deformed; this can be combated by the rigidity of the leaflets. Reducing rigidity would reduce the cost of deformation energy and facilitate

651 *nucleation. Rigidity is often increased by PL saturation, cholesterol, and possibly sterol*  
652 *esters, which would disfavor nucleation. Another membrane input is surface tension;*  
653 *increasing the bilayer surface tension, by pulling or stretching on the bilayer, would disfavor*  
654 *bilayer deformation and nucleation [40,41]. In vitro, LD nucleation requires low bilayer*  
655 *tension.*  
656  
657  
658

## Figure legends

**Figure 1:** a) Schematic illustration of the esterification of diacylglycerol or cholesterol by diacylglycerol O-acyltransferase 1 (DGAT1), or acyl-coenzyme A: cholesterol acyltransferase 1/sterol O-acyltransferase 1 (ACAT1/SOAT1), or membrane-bound O-acyl transferase (MBOAT). The synthesized neutral lipid exits the enzyme through a lateral gate facing the bilayer hydrophobic core. b) Molecular structures of triolein and sterol oleate, the major triglycerides and sterol esters in neutral lipid stores. c) Examples of other neutral lipids with ester functions. d) Examples of other neutral lipids with non-ester functions.

**Figure 2.** Schematic illustration of the condensation of triglycerides (TG) in protein-free (pf), seipin deficient (S-), and in seipin containing conditions (S+). Increasing the concentration of TG per phospholipids, [TG/PL] leads to the formation of transient clusters that are bigger steadier for larger TG concentrations. Nucleation, i.e. TG condensation, happens at a critical concentration around ~5% in protein-free conditions. Proteins, in particular seipin, might decrease the critical nucleation concentration. ER: endoplasmic reticulum, LD: lipid droplet, AH: amphipathic helix, Plin1: perilipin 1, LDAF1: lipid droplet factor 1.

**Figure 3.** a) Illustration of the evolution of TG LD nucleation frequency and LD size pending on ER tubule/sheet ratio. b) Illustration of the enrichment of seipin in tubules vs. sheets. In seipin deficiency, more TG LD nucleation events happen at tubules. c) Evolution of nucleation frequency pending on the seipin/tubule ratio. At low seipin dose, a lipodystrophic phenotype is observed. d) In a single cell, seipin is trapped at the nuclear envelope membrane where a normal LD biogenesis phenotype is observed. In the rest of the cell ("periphery"), a seipin deficient and lipodystrophic phenotype is observed. A critical density of seipin is required throughout the cell to prevent the undesired lipodystrophic phenotype. Figure adapted from Figure 7 of Salo et al. *Developmental Cell* 2019 [31].





