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Cell biology of lipid droplets

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Lipid storage has attracted much attention in the past years, both by the broader public and the biomedical scientific community. Driven by concerns about the obesity epidemic that affects most industrialized countries and even substantial parts of the population in less and least developed countries, work from researchers of many disciplines has shed light on the genetics, the physiology, and the cellular mechanisms of fat accumulation. This review focuses on the actual organelle of fat deposition, the lipid droplet (LD), and on the recent progress in mechanistic understanding of processes like LD biogenesis, LD growth and degradation, protein targeting to LDs and LD fusion.

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Introduction

Lipid droplets (LDs) are intracellular and cytoplasmic structures, which store neutral lipids in their core. In most tissues, triglycerides and sterol ester are the most abundant constituents of the core, but other endogenous neutral lipids like free cholesterol and retinol ester, and xenobiotic hydrophobic compounds such as polycyclic aromatic hydrocarbons, are also found in the droplet core. The core is surrounded by a monolayer of polar lipids [1] with some attached or embedded proteins. Whether droplets have, beyond this basic structure, any internal organization, is a matter of debate. By electron microscopy, some LDs display an internal structure, which ranges from shell-like organization of lipid layers [2] to the apparent presence of internal membrane compartments [3]. By immunofluorescence microscopy or life cell imaging with fluorescently tagged LD surface proteins, no internal structures have been observed so far, suggesting that they might be generated by EM sample preparation. Yet, it should be noticed that reports of internal LD structure are mostly based on observations in macrophages and leucocyte LDs, while most other studies on LDs were done in fibroblasts, hepatocytes, and adipocytes. It might well be that particularly macrophages and neutrophils with their intense phagocytic activity contain hybrid compartments of phospholipid-rich lamellar bodies and triglyceride-rich LDs [4].

Formation and growth of lipid droplets

Unlike most other organelles, lipid droplets are not formed by growth and fission of existing droplets but seemingly can be formed *de novo*. Despite some recent efforts, we have to state that the site of origin, the mechanism, and the machinery involved are unknown. LD *de novo* formation has been visualized in cells that were kept under delipidated conditions and re-fed with free fatty acid to induce rapid formation of LDs. Both observation of a fluorescent LD protein [5] and of fluorescent fatty acid [6••] show concentration of LD components in the ER or its direct vicinity within 5–15 min, followed by the rapid formation of droplets. Whether the initial event of lipid accumulation takes place between the two leaflets of the ER membrane or on its cytoplasmic surface is beyond the resolution of light microscopy. A frequently cited hypothesis [7] favors the first possibility, on the basis of some ultrastructural observations that suggest a continuity of the LD surface monolayer and the cytoplasmic leaflet of adjacent ER cisternae [8,9]. This hypothesis elegantly explains the origin of the LD surface monolayer, which would be formed from the cytoplasmic leaflet of the ER membrane, and the targeting of some ER membrane proteins to the forming LD via the membrane continuity. Nonetheless, in the absence of direct evidence, the model remains speculative, and should be used with caution as a basis of further hypotheses [10]. To support this model, time-resolved ultrastructural studies focusing on the first minutes of LD formation would be extremely informative, ideally by correlative fluorescence/electron microscopy to capture sites of actual droplet biogenesis. An alternative model favors formation of droplets at specialized sites of the cytoplasmic surface of the ER. These sites surround the forming droplets in an egg-cup-like manner and contain high concentrations of the LD PAT protein adipophilin [11•]. It has to be stated that the data presented do not show freshly formed droplets but droplets that are already several hundreds of nanometers in diameter. The model also does not offer an explanation how initial triglyceride and surface lipid would be transferred from the ER and be organized to form an LD. A further concern is the fact that adipophilin, which is proposed to have an important role in the process, is not essential for LD formation as shown by a adipophilin knockout mouse [12] and by the

existence of very similar LDs in yeast, which does not have any protein of the PAT family.

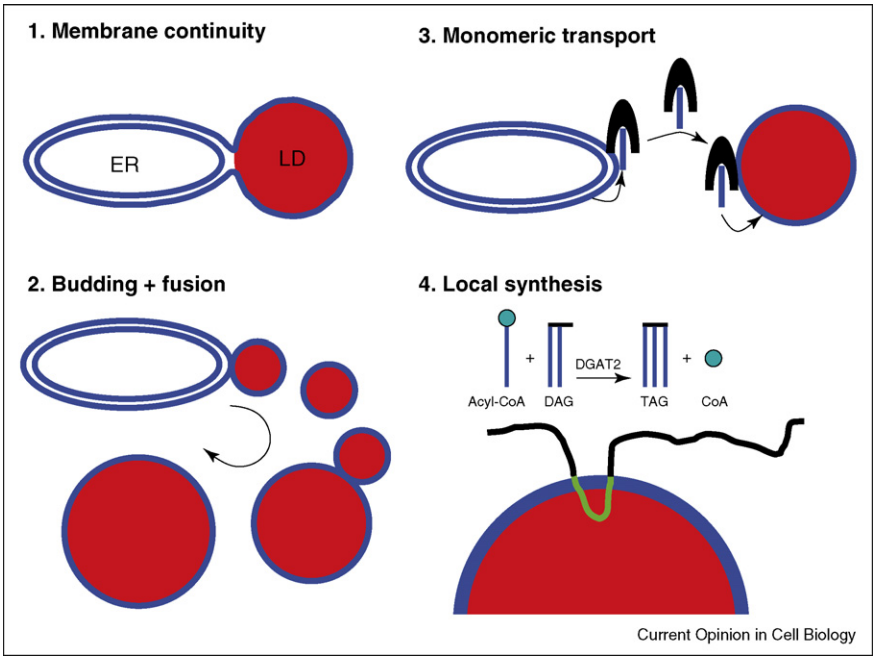
Apart from the initial step of droplet formation, several recent publications have identified proteins that act in a regulatory way to control LD formation. This includes PLD1 and ERK2 [13], the BARS protein [14], FSP27 [15], OXPAT [16], Prp19p [17], and FIT1/2 [18].

Under continuous supply of fatty acid, the nascent lipid droplets continue growing until they reach a final size that depends on the cell type and typically ranges between 1 and 20 μm. The understanding of the mechanism of this growth is again intimately linked to understanding LD formation and LD homotypic fusion. Most of the biosynthetic enzymatic activities for triglyceride and sterol ester formation reside to microsomal cell fractions, or more specifically, to the ER. The ER is the key organelle for membrane lipid biosynthesis, which is identical with neutral storage lipid biosynthesis except for the final acylation to yield triglyceride or sterol ester, catalyzed by DGAT1 and ACAT1. These two final activities are found at the ER, which enables neutral lipid production for secretion along the ER–Golgi secretory pathway as a lipoprotein particle. If the ER was the only site of triglyceride synthesis, droplets could grow by various mechanisms (Figure 1): first, permanent connection to the ER by membrane continuities; second, continuous production of nascent LDs followed by homotypic fusion; or third, monomeric transport of TG by a carrier mechanism. Currently, none of these possibilities is strongly supported by experimental evidence. Membrane continuities were only sporadically found; a TG carrier mechanism is not known, and fusion is, despite a recent report that proposes a mechanistic basis [19•], a rare process that is difficult to detect at all in most types of cultured cells [20]. Two recent studies now offer an alternative: Fujimoto *et al.* demonstrated that isolated LDs could synthesize neutral lipids [21•]. Kuerschner *et al.* presented a detailed study on kinetics and localization of TG biogenesis which demonstrated that DAG, the metabolic precursor of TG, accumulates on LDs and is used for TG synthesis at the surface of LDs, catalyzed by DGAT2, which is the key enzyme for the production of storage triglyceride [22,23]. This elegantly explains how droplets can grow in the apparent absence of a physical connection to the ER and obviates the need of repetitive fusion cycles with nascent droplets.

Protein targeting

Like any other membrane organelle, the LD surface monolayer also contains a characteristic set of proteins.

Figure 1



Pathways of lipid and protein targeting to LDs. (1) By continuity of the LD surface monolayer with the outer leaflet of the ER membrane, both lipid and proteins would travel from their site of synthesis at the ER to the LD. Topologically, the LD would be a subdomain of the ER. This is different in the other models: in (2), a small droplet generated like in (1), would bud from the ER, travel through the cytosol and fuse with an existing larger droplet to deliver both surface and core components. Also, surface components could travel by monomeric transport, facilitated by one of the numerous cytoplasmic lipid-binding proteins. Finally, both core and surface lipids may be synthesized directly on the droplet, exemplified by the formation of triglyceride, catalyzed by DGAT2. Note that none of these scenarios is exclusive, they may contribute to different degrees to transport of different substrates.

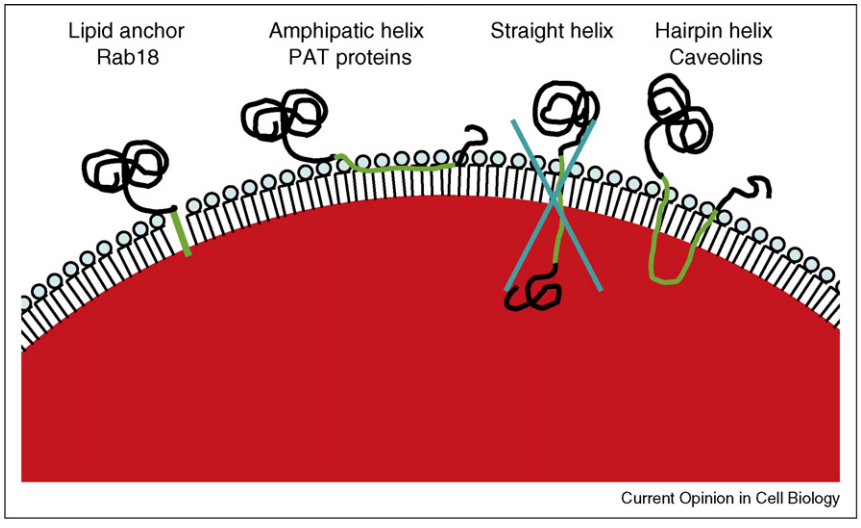
By mass spectrometrical analysis of LD preparations from various cell lines and tissues, recent work from several laboratories [3,24–29,30•] has identified two groups of proteins that dominate a proteome of a typical size of about 30–50 major components. The first group is the PAT family of peripheral membrane proteins, named for the first identified members perilipin, adipophilin and TIP47, which serve structural and regulatory purposes [31]. The other major group consists of enzymes of lipid metabolism, that is, acyl-CoA synthetases, lipases that act on triglyceride, and enzymes of sterol biogenesis, which cluster into very early (squalene epoxidase, lanosterol synthase) and late (17-hydroxysteroid dehydrogenases, NSDHL) steps of the pathway.

From the structural point of view, the LD proteome consists of three classes (Figure 2): peripherally associated proteins such as the PAT family members, lipid-anchored proteins of the small GTPase type, and monotopic integral membrane proteins. Polytopic membrane proteins appear in LD proteome lists, but for none of them localization to LDs was confirmed by immunofluorescence microscopy or live cell imaging, suggesting that they represent contamination with other membranes. This lack of polytopic proteins is a logical consequence of the unusual monolayer organization of the LD surface, which does not provide the luminal aqueous compartment necessary to solubilize the hydrophilic loops that connect the transmembrane regions of polytopic proteins. The LD integral monotopic membrane proteins share a typical organization, exemplified in Figure 3 by five well-characterized proteins: NSDHL [32,33] and DGAT2 [6••,34], both enzymes of lipid metabolism, and the three caveolin proteins [35–37]. All of them have a long hydrophobic region that typically extends over 30–40 amino acids and contains flexible regions with many residues that destabilize a regular straight alpha helix. It seems to be this overall organization, not distinct sequence motifs, which governs the targeting to the LD [38]. Also, none of these proteins has a bona fide signal sequence for sec61-dependent ER translocation (Figure 3), as found in typical single span transmembrane proteins. As a consequence of the long hydrophobic stretch and the lack of a signal sequence, both termini of the LD proteins face the cytoplasm to result in a hairpin topology, which was experimentally shown for NSDHL [33], DGAT2 [34], and the caveolin [39] proteins. A further common property of these five and other LD proteins is the dynamic shift of localization between LD and ER [6••,32] or plasma membrane [40,41], induced by a change in cellular supply of fatty acid or cholesterol. The lipid-sensing mechanism and the transport pathway behind this apparently bi-directional mobility remain unknown, but is logically connected to the question of the origin and formation of droplets. If droplets bud from the ER, it should involve flow over membrane continuities; otherwise, proteins might also be shuttled in a soluble form, probably stabilized by chaperones.

LD degradation/lipolysis

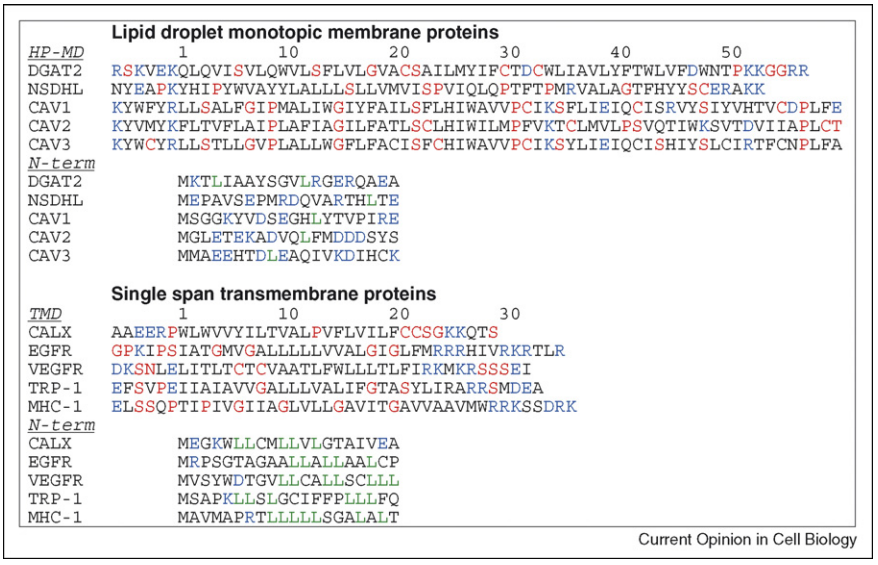
The opposite of LD growth is the process of shrinkage, commonly called lipolysis. This has been studied in mechanistic detail in the adipocyte model cell line 3T3-L1 and recently summarized in an excellent review [31]. Strategically, the cell employs the same principles as in the growth of droplets. The insoluble triglyceride is not transported within the cell but is converted to soluble products, that is, fatty acids and monoacylglycerol,

Figure 2



Attachments of proteins to the surface of LDs. The hydrophobic LD core (red) is surrounded by a monolayer of phospholipids, to which proteins can attach by lipid anchors, amphipatic helices or hairpin helices. Polytopic proteins are not found on LDs, because their hydrophilic domains (black) cannot be integrated into the LD core.

Figure 3



LD monotopic membrane proteins have long hydrophobic sequences with regular interspaced helix-braking amino acids and lack N-terminal hydrophobic leucine-rich signal sequences. For comparison, five single-span transmembrane proteins with shorter TMD and a characteristic N-terminal signal sequence are shown. Position 1 indicates the first residue of the hydrophobic stretch or the N-terminal methionine. Color code in the membrane domains: Blue - charged residues, Red - helix braking (P, G, S, C). Color code for N-termini: Blue - charged residues, Green - leucine. Sequences are taken from Swissprot with the following accession numbers: DGAT2 Q96PD7, NSDHL Q15738, CAV1 Q03135, CAV2 P51636, CAV3 P56539, CALX P27842, EGFR P00533, VEGFR P17948, TRP-1 P17643, MHC-1 P30433.

directly at the LD surface by the sequential action of two lipases, ATGL and HSL [42]. Perilipin, one of the PAT family proteins [31] plays a key role in the organization and regulation of this process. Perilipin is preferentially found at large, 'mature' lipid droplets [43]. An external lipolytic stimulus activates the adrenergic receptor system to trigger phosphorylation of perilipin and other proteins by the cAMP-dependent kinase PKA, which gives rise to two cellular reactions. First, droplets disperse over the cellular volume and large droplets fragment into smaller ones, which is controlled by perilipin phosphorylation at several serine residues [44,45]. This morphological rearrangement largely increases the accessible droplet surface and enhances the second reaction, which is the dynamic recruitment of ATGL, its regulator CGI-58 [46,47•], and HSL to a subpopulation of lipid droplets [48–50], followed by the degradation of the TG core. A further component that probably plays a regulatory role in lipolysis is the small GTPase Rab18. It localizes to LDs [51•] where it usually covers a subpopulation of droplets [52•]. Lipolytic stimulation increases LD recruitment of Rab18. Rab18 recruitment to LDs displaces ADRP, which is a stimulator of droplet formation, and induces contact with ER membranes, which might facilitate utilization of lipids released during lipolysis.

With progression of lipolysis, not only the TG but also the entire organelle disappears, raising the question of the fate of LD proteins. At least LD caveolin, and probably more of the hairpin monotopic LD proteins, relocate to other membranes [40]. Other components, notably the PAT proteins ADRP and perilipin, are subject to ubiquitin-dependent proteasomal degradation [53–55], which also affects excess apolipoprotein B that is degraded at the LD surface [56]. The regulatory potential of LD protein degradation, in analogy to the regulated degradation of the central enzyme of sterol metabolism, HMGR, will be an important subject of future studies.

Since lipolysis is probably the best characterized part of the droplet life cycle, it can be used to exemplify the ancient nature and the high degree of conservation in the system. Drosophila has the perilipin homolog LSD2, which controls both triglyceride storage [57] and LD mobility [58]. The human ATGL lipase has a drosophila homolog with the same function [59], and drosophila lipolysis is also regulated by a hormonal signaling pathway [60•]. In yeast, which does not have a perilipin homolog, still the lipase is conserved [61], as is the complete glycerolipid biosynthetic pathway [62] which actually was the starting point for the identification of several of the corresponding mammalian enzymes.

Fusion

Lipid droplet homotypic fusion still is a controversial subject. During differentiation from a fibroblast type precursor to an adipocyte phenotype, the cell line 3T3-L1 remodels its LD pool from 100 or more small droplets

to often less than 10 large droplets. It is widely assumed that homotypic fusion is part of this process, but since the differentiation takes about two weeks, no imaging data that show this fusion are actually available. In differentiated 3T3-L1 cells, it was reported that fusion is not visible under conditions of active lipid synthesis [20], consistent with a lack of well-documented fusion events in the literature in general and also several failed attempts to observe fusion from this author's laboratory. There is one report that in the cell line NIH 3T3 droplets grow in size by a mechanism called by the authors 'complex formation' [63], but the data presented did not allow to unequivocally distinguish between permanent adhesion of droplets, which is frequently observed in many cell types, and actual fusion. The same experimental system was recently used [19•] to study mechanistic details of the complex formation/fusion-event. The authors concluded that the three SNARE proteins SNAP-23, syntaxin-5, and VAMP4 would mediate droplet fusion in analogy to SNARE protein action in bilayer membrane fusion events. Although this represents a stimulating finding, rigorous experimentation will be needed to evaluate it in other cell types and in particular in systems reconstituted from purified components [64]. It would also be useful to study fusion in systems, where it may be more easily visualized, as recently proposed for the remodeling of the droplet pool in 3T3-L1 cells upon treatment with the adrenergic antagonist propranolol [31].

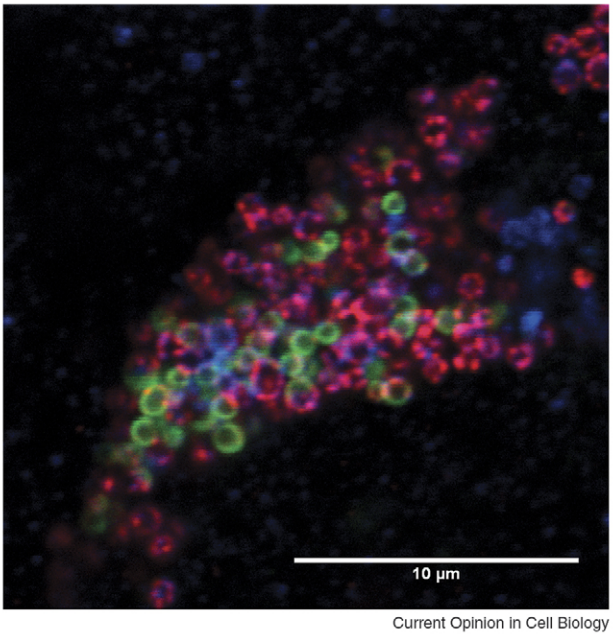
An interesting aspect of fusion is the question — why it is such a rare event. Several cell types contain cluster consisting of some tens to hundreds of densely packed individual LDs. In these clusters, there are obvious signs of mechanical strain that presses droplets onto each other resulting in distortion of their normal perfect round shape. In our hands, even in those clusters, continuous imaging over periods of around 60 min usually fails to reveal fusion events. This is remarkable, given that droplets have only a monolayer membrane, which reduces fusion to a single step, the merger of the monolayers. Hemifusion intermediates that form in bilayer fusion [65] cannot form in LD fusion, which therefore should proceed more readily than bilayer fusion events. Also, although quantitative data are lacking so far, the low amounts of protein that are recovered from LD preparations suggest that the LD surface is not densely covered with proteins that could have antifusogenic activity. It seems that the lipid composition of the LD monolayer may play a key role in the inhibition of spontaneous fusion. Lipid analysis from isolated droplets by TLC separation followed by charring [1] or detecting radioactive or fluorescently labeled lipids [6••] suggest that PC is by far the most abundant constituent of this surface, much in contrast to other cytoplasmic organelle surfaces that contain abundant PE, PS, and PI [66]. Two recent studies have shown that the size of LDs crucially depends on the action of the protein Seipin/Fld1p [67••,68••]. LDs from yeast lacking Fld1p showed increased spontaneous fusion, concomitant with a slight shift toward shorter species in the balance of side chain length in the total phospholipid pool [68••]. How this change in total lipid composition is reflected in LD surface lipid composition and how the lipid chain length would regulate fusion behavior needs still to be addressed.

Conclusion and outlook

One major future challenge in LD cell biology is the understanding of the dynamics of the complete organelle as well as of its single constituents. This will be supported by technological progress in live cell imaging such as the use of novel fluorescent lipids [69] that allow monitoring of lipid flow to LDs in living cells [6••]. A further step is the use of label free imaging by nonlinear optical techniques. Owing to the large differences to the surrounding cytoplasm regarding physico-chemical properties and composition, LDs are particularly favorable for imaging by third harmonic generation (THG) [70] and coherent anti-Stokes Raman scattering (CARS) microscopy [71–73]. Both methods have in common deep penetration into specimen, which allows imaging in tissues, and low phototoxicity to allow long-term imaging of LD dynamics in living cells [74].

The other major challenge is understanding of the highly regulated, integrated, and strongly compartmentalized

Figure 4



The LD pool is not homogenous. Although apparently equal in size and shape, neighboring LDs can vary in metabolic activity [6••] and in protein content, as shown in this immunofluorescence picture from a human A431 cell, which was stained for three different LD surface proteins (AUP1, blue; ORP2, red; Rab18, green).

cellular network of metabolism, signaling, and trafficking that leads to LD formation or degradation. Even within one cell, droplets can be different in metabolic activity and protein content (Figure 4). With the first results of large-scale genetic analysis in yeast [67••,68••] and insect cells [75••] we know the identity of many of the network components. Hopefully, cell biology and biochemistry will soon be able to elucidate their functions in mechanistic detail.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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

2. Robenek MJ, Severs NJ, Schlattmann K, Plenz G, Zimmer KP, Troyer D, Robenek H: **Lipids partition caveolin-1 from ER membranes into lipid droplets: updating the model of lipid droplet biogenesis**. *FASEB J* 2004, **18**:866-868.

3. Wan HC, Melo RC, Jin Z, Dvorak AM, Weller PF: **Roles and origins of leukocyte lipid bodies: proteomic and ultrastructural studies**. *FASEB J* 2007, **21**:167-178.

4. Robenek H, Schmitz G: **Ca++ antagonists and ACAT inhibitors promote cholesterol efflux from macrophages by different mechanisms. II. Characterization of intracellular morphologic changes**. *Arteriosclerosis* 1988, **8**:57-67.

5. Turro S, Ingelmo-Torres M, Estanyol JM, Tebar F, Fernandez MA, Albor CV, Gaus K, Grewal T, Enrich C, Pol A: **Identification and characterization of associated with lipid droplet protein. 1. A novel membrane-associated protein that resides on hepatic lipid droplets**. *Traffic* 2006, **7**:1254-1269.

6. •• Kuerschner L, Moessinger C, Thiele C: **Imaging of lipid biosynthesis: how a neutral lipid enters lipid droplets**. *Traffic* 2008, **9**:338-352.
By a combination of life cell imaging and kinetic biochemical analysis, this study shows that triglyceride is formed on the surface of LDs, catalyzed by DGAT2. This implies that LDs grow independent of fusion events by local synthesis of triglyceride.

7. Murphy DJ, Vance J: **Mechanisms of lipid-body formation**. *Trends Biochem Sci* 1999, **24**:109-115.

8. Stein O, Stein Y: **Lipid synthesis, intracellular transport, and secretion. II. Electron microscopic radioautographic study of the mouse lactating mammary gland**. *J Cell Biol* 1967, **34**:251-263.

9. Blanchette-Mackie EJ, Dwyer NK, Barber T, Coxey RA, Takeda T, Rondinone CM, Theodorakis JL, Greenberg AS, Londos C: **Perilipin is located on the surface layer of intracellular lipid droplets in adipocytes**. *J Lipid Res* 1995, **36**:1211-1226.

10. Ploegh HL: **A lipid-based model for the creation of an escape hatch from the endoplasmic reticulum**. *Nature* 2007, **448**:435-438.

11. • Robenek H, Hofnagel O, Buers I, Robenek MJ, Troyer D, Severs NJ: **Adipophilin-enriched domains in the ER membrane are sites of lipid droplet biogenesis**. *J Cell Sci* 2006, **119**:4215-4224.
Formulates the hypothesis of LD formation at specialized sites on the cytoplasmic surface of the ER membrane.

12. Chang BH, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, Chan L: **Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein**. *Mol Cell Biol* 2006, **26**:1063-1076.

13. Andersson L, Bostrom P, Ericson J, Rutberg M, Magnusson B, Marchesan D, Ruiz M, Asp L, Huang P, Frohman MA *et al.*: **PLD1 and ERK2 regulate cytosolic lipid droplet formation**. *J Cell Sci* 2006, **119**:2246-2257.

14. Bartz R, Seemann J, Zehmer JK, Serrero G, Chapman KD, Anderson RG, Liu P: **Evidence that mono-ADP-ribosylation of CtBP1/BARS regulates lipid storage**. *Mol Biol Cell* 2007, **18**:3015-3025.

15. Puri V, Konda S, Ranjit S, Aouadi M, Chawla A, Chouinard M, Chakladar A, Czech MP: **Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage**. *J Biol Chem* 2007, **282**:34213-34218.

16. Wolins NE, Quaynor BK, Skinner JR, Tzekov A, Croce MA, Gropler MC, Varma V, Yao-Borengasser A, Rasouli N, Kern PA *et al.*: **OXPAT/PAT-1 is a PPAR-induced lipid droplet protein that promotes fatty acid utilization**. *Diabetes* 2006, **55**:3418-3428.

17. Cho SY, Shin ES, Park PJ, Shin DW, Chang HK, Kim D, Lee HH, Lee JH, Kim SH, Song MJ *et al.*: **Identification of mouse Prp19p as a lipid droplet-associated protein and its possible involvement in the biogenesis of lipid droplets**. *J Biol Chem* 2007, **282**:2456-2465.

18. Kadereit B, Kumar P, Wang WJ, Miranda D, Snapp EL, Severina N, Torregroza I, Evans T, Silver DL: **Evolutionarily conserved gene family important for fat storage**. *Proc Natl Acad Sci U S A* 2008, **105**:94-99.

19. • Bostrom P, Andersson L, Rutberg M, Perman J, Lidberg U, Johansson BR, Fernandez-Rodriguez J, Ericson J, Nilsson T, Boren J *et al.*: **SNARE proteins mediate fusion between cytosolic lipid droplets and are implicated in insulin sensitivity**. *Nat Cell Biol* 2007, **9**:1286-1293.
Proposes a mechanistic basis for homeotypic fusion of LDs as a SNARE-mediated process.

20. Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A, Bickel PE: **S3-12, adipophilin, and TIP47 package lipid in adipocytes**. *J Biol Chem* 2005, **280**:19146-19155.

21. • Fujimoto Y, Itabe H, Kinoshita T, Homma KJ, Onoduka J, Mori M, Yamaguchi S, Makita M, Higashi Y, Yamashita A *et al.*: **Involvement of ACSL in local synthesis of neutral lipids in cytoplasmic lipid droplets in human hepatocyte HuH7**. *J Lipid Res* 2007, **48**:1280-1292.
Important study to demonstrate localized synthesis of neutral lipid on isolated lipid droplets.

22. Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, Elias PM, Farese RV Jr: **Lipopenia and skin barrier abnormalities in DGAT2-deficient mice**. *J Biol Chem* 2004, **279**:11767-11776.

23. Levin MC, Monetti M, Watt MJ, Sajan MP, Stevens RD, Bain JR, Newgard CB, Farese RV Sr, Farese RV Jr: **Increased lipid accumulation and insulin resistance in transgenic mice expressing DGAT2 in glycolytic (type II) muscle**. *Am J Physiol Endocrinol Metab* 2007, **293**:E1772-E1781.

24. Athenstaedt K, Zweytick D, Jandrositz A, Kohlwein SD, Daum G: **Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae***. *J Bacteriol* 1999, **181**:6441-6448.

25. Liu P, Ying Y, Zhao Y, Mundy DI, Zhu M, Anderson RG: **Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic**. *J Biol Chem* 2004, **279**:3787-3792.

26. Brasaemle DL, Dolios G, Shapiro L, Wang R: **Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes**. *J Biol Chem* 2004, **279**:46835-46842.

27. Fujimoto Y, Itabe H, Sakai J, Makita M, Noda J, Mori M, Higashi Y, Kojima S, Takano T: **Identification of major proteins in the lipid**

droplet-enriched fraction isolated from the human hepatocyte cell line HuH7. *Biochim Biophys Acta* 2004, **1644**:47-59.

28. Beller M, Riedel D, Jansch L, Dieterich G, Wehland J, Jackle H, Kuhnlein RP: **Characterization of the Drosophila lipid droplet subproteome**. *Mol Cell Proteomics* 2006, **5**:1082-1094.

29. Sato S, Fukasawa M, Yamakawa Y, Natsume T, Suzuki T, Shoji I, Aizaki H, Miyamura T, Nishijima M: **Proteomic profiling of lipid droplet proteins in hepatoma cell lines expressing hepatitis C virus core protein**. *J Biochem* 2006, **139**:921-930.

30. • Cermelli S, Guo Y, Gross SP, Welte MA: **The lipid-droplet proteome reveals that droplets are a protein-storage depot**. *Curr Biol* 2006, **16**:1783-1795.
Interesting study that proposes that LDs serve as a site of protein storage in the development of the drosophila embryo.

31. Brasaemle DL: **Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis**. *J Lipid Res* 2007, **48**:2547-2559.

32. Ohashi M, Mizushima N, Kabeya Y, Yoshimori T: **Localization of mammalian NAD(P)H steroid dehydrogenase-like protein on lipid droplets**. *J Biol Chem* 2003, **278**:36819-36829.

33. Caldas H, Herman GE: **NSDHL, an enzyme involved in cholesterol biosynthesis, traffics through the Golgi and accumulates on ER membranes and on the surface of lipid droplets**. *Hum Mol Genet* 2003, **12**:2981-2991.

34. Stone SJ, Levin MC, Farese RV Jr: **Membrane topology and identification of key functional amino acid residues of murine acyl-CoA:diacylglycerol acyltransferase-2**. *J Biol Chem* 2006, **281**:40273-40282.

35. Fujimoto T, Kogo H, Ishiguro K, Tauchi K, Nomura R: **Caveolin-2 is targeted to lipid droplets, a new ''membrane domain'' in the cell**. *J Cell Biol* 2001, **152**:1079-1085.

36. Ostermeyer AG, Paci JM, Zeng Y, Lublin DM, Munro S, Brown DA: **Accumulation of caveolin in the endoplasmic reticulum redirects the protein to lipid storage droplets**. *J Cell Biol* 2001, **152**:1071-1078.

37. Pol A, Luetterforst R, Lindsay M, Heino S, Ikonen E, Parton RG: **A caveolin dominant negative mutant associates with lipid bodies and induces intracellular cholesterol imbalance**. *J Cell Biol* 2001, **152**:1057-1070.

38. Ostermeyer AG, Ramcharan LT, Zeng Y, Lublin DM, Brown DA: **Role of the hydrophobic domain in targeting caveolin-1 to lipid droplets**. *J Cell Biol* 2004, **164**:69-78.

39. Dupree P, Parton RG, Raposo G, Kurzchalia TV, Simons K: **Caveolae and sorting in the trans-Golgi network of epithelial cells**. *EMBO J* 1993, **12**:1597-1605.

40. Pol A, Martin S, Fernandez MA, Ferguson C, Carozzi A, Luetterforst R, Enrich C, Parton RG: **Dynamic and regulated association of caveolin with lipid bodies: modulation of lipid body motility and function by a dominant negative mutant**. *Mol Biol Cell* 2004, **15**:99-110.

41. Le Lay S, Hajduch E, Lindsay MR, Le Liepvre X, Thiele C, Ferre P, Parton RG, Kurzchalia T, Simons K, Dugail I: **Cholesterol-induced caveolin targeting to lipid droplets in adipocytes: a role for caveolar endocytosis**. *Traffic* 2006, **7**:549-561.

42. Zechner R, Strauss JG, Haemmerle G, Lass A, Zimmermann R: **Lipolysis: pathway under construction**. *Curr Opin Lipidol* 2005, **16**:333-340.

43. Wolins NE, Brasaemle DL, Bickel PE: **A proposed model of fat packaging by exchangeable lipid droplet proteins**. *FEBS Lett* 2006, **580**:5484-5491.

44. Marcinkiewicz A, Gauthier D, Garcia A, Brasaemle DL: **The phosphorylation of serine 492 of perilipin a directs lipid droplet fragmentation and dispersion**. *J Biol Chem* 2006, **281**:11901-11909.

45. Miyoshi H, Perfield JW II, Souza SC, Shen WJ, Zhang HH, Stancheva ZS, Kraemer FB, Obin MS, Greenberg AS: **Control of adipose triglyceride lipase action by serine 517 of perilipin A globally regulates protein kinase A-stimulated lipolysis in adipocytes**. *J Biol Chem* 2007, **282**:996-1002.

46. Yamaguchi T, Omatsu N, Matsushita S, Osumi T: **CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin–Dorfman syndrome**. *J Biol Chem* 2004, **279**:30490-30497.

47. • Lass A, Zimmermann R, Haemmerle G, Riederer M, Schoiswohl G, Schweiger M, Kienesberger P, Strauss JG, Gorkiewicz G, Zechner R: **Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin–Dorfman Syndrome**. *Cell Metab* 2006, **3**:309-319.
Identifies CGI-58 as a potent regulator of the triglyceride lipase ATGL.

48. Moore HP, Silver RB, Mottillo EP, Bernlohr DA, Granneman JG: **Perilipin targets a novel pool of lipid droplets for lipolytic attack by hormone-sensitive lipase**. *J Biol Chem* 2005, **280**:43109-43120.

49. Miyoshi H, Souza SC, Zhang HH, Strissel KJ, Christoffolete MA, Kovsan J, Rudich A, Kraemer FB, Bianco AC, Obin MS *et al.*: **Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms**. *J Biol Chem* 2006, **281**:15837-15844.

50. Granneman JG, Moore HP, Granneman RL, Greenberg AS, Obin MS, Zhu Z: **Analysis of lipolytic protein trafficking and interactions in adipocytes**. *J Biol Chem* 2007, **282**:5726-5735.

51. • Ozeki S, Cheng J, Tauchi-Sato K, Hatano N, Taniguchi H, Fujimoto T: **Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane**. *J Cell Sci* 2005, **118**:2601-2611.
See annotation to [52•].

52. • Martin S, Driessen K, Nixon SJ, Zerial M, Parton RG: **Regulated localization of Rab18 to lipid droplets: effects of lipolytic stimulation and inhibition of lipid droplet catabolism**. *J Biol Chem* 2005, **280**:42325-42335.
Along with [51•], this study demonstrates specific localization of Rab18 to LDs and its probable relevance for LD association with the ER and regulation of lipolysis.

53. Xu G, Sztalryd C, Lu X, Tansey JT, Gan J, Dorward H, Kimmel AR, Londos C: **Post-translational regulation of adipose differentiation-related protein by the ubiquitin/proteasome pathway**. *J Biol Chem* 2005, **280**:42841-42847.

54. Masuda Y, Itabe H, Odaki M, Hama K, Fujimoto Y, Mori M, Sasabe N, Aoki J, Arai H, Takano T: **ADRP/adipophilin is degraded through the proteasome-dependent pathway during regression of lipid-storing cells**. *J Lipid Res* 2006, **47**:87-98.

55. Xu G, Sztalryd C, Londos C: **Degradation of perilipin is mediated through ubiquitination–proteasome pathway**. *Biochim Biophys Acta* 2006, **1761**:83-90.

56. Ohsaki Y, Cheng J, Fujita A, Tokumoto T, Fujimoto T: **Cytoplasmic lipid droplets are sites of convergence of proteasomal and autophagic degradation of apolipoprotein B**. *Mol Biol Cell* 2006, **17**:2674-2683.

57. Gronke S, Beller M, Fellert S, Ramakrishnan H, Jackle H, Kuhnlein RP: **Control of fat storage by a Drosophila PAT domain protein**. *Curr Biol* 2003, **13**:603-606.

58. Welte MA, Cermelli S, Griner J, Viera A, Guo Y, Kim DH, Gindhart JG, Gross SP: **Regulation of lipid-droplet transport by the perilipin homolog LSD2**. *Curr Biol* 2005, **15**:1266-1275.

59. Gronke S, Mildner A, Fellert S, Tennagels N, Petry S, Muller G, Jackle H, Kuhnlein RP: **Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila**. *Cell Metab* 2005, **1**:323-330.

60. • Gronke S, Muller G, Hirsch J, Fellert S, Andreou A, Haase T, Jackle H, Kuhnlein RP: **Dual lipolytic control of body fat storage and mobilization in Drosophila**. *PLoS Biol* 2007, **5**:e137.
Shows that lipolysis in Drosophila is controlled by at least two independent convergent pathways.

61. Kurat CF, Natter K, Petschnigg J, Wolinski H, Scheuringer K, Scholz H, Zimmermann R, Leber R, Zechner R, Kohlwein SD: **Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast**. *J Biol Chem* 2006, **281**:491-500.

62. Daum G, Wagner A, Czabany T, Athenstaedt K: **Dynamics of neutral lipid storage and mobilization in yeast**. *Biochimie* 2007, **89**:243-248.

63. Bostrom P, Rutberg M, Ericsson J, Holmdahl P, Andersson L, Frohman MA, Boren J, Olofsson SO: **Cytosolic lipid droplets increase in size by microtubule-dependent complex formation**. *Arterioscler Thromb Vasc Biol* 2005, **25**:1945-1951.

64. Sollner TH: **Lipid droplets highjack SNAREs**. *Nat Cell Biol* 2007, **9**:1219-1220.

65. Chernomordik LV, Zimmerberg J, Kozlov MM: **Membranes of the world unite!** *J Cell Biol* 2006, **175**:201-207.

66. van Meer G, Voelker DR, Feigenson GW: **Membrane lipids: where they are and how they behave**. *Nat Rev Mol Cell Biol* 2008, **9**:112-124.

67. •• Szymanski KM, Binns D, Bartz R, Grishin NV, Li WP, Agarwal AK, Garg A, Anderson RG, Goodman JM: **The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology**. *Proc Natl Acad Sci U S A* 2007, **104**:20890-20895.

68. •• Fei W, Shui G, Gaeta B, Du X, Kuerschner L, Li P, Brown AJ, Wenk MR, Parton RG, Yang H: **Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast**. *J Cell Biol* 2008, **180**:473-482.
These two large-scale genetic studies of LD formation in yeast demonstrate the conservation of the basic machinery for LD formation from yeast to man.

69. Kuerschner L, Ejsing CS, Ekroos K, Shevchenko A, Anderson KI, Thiele C: **Polyene-lipids: a new tool to image lipids**. *Nat Methods* 2005, **2**:39-45.

70. Debarre D, Supatto W, Pena AM, Fabre A, Tordjmann T, Combettes L, Schanne-Klein MC, Beaurepaire E: **Imaging lipid bodies in cells and tissues using third-harmonic generation microscopy**. *Nat Methods* 2006, **3**:47-53.

71. Nan X, Cheng JX, Xie XS: **Vibrational imaging of lipid droplets in live fibroblast cells with coherent anti-Stokes Raman scattering microscopy**. *J Lipid Res* 2003, **44**:2202-2208.

72. Hellerer T, Axang C, Brackmann C, Hillertz P, Pilon M, Enejder A: **Monitoring of lipid storage in *Caenorhabditis elegans* using coherent anti-Stokes Raman scattering (CARS) microscopy**. *Proc Natl Acad Sci U S A* 2007, **104**:14658-14663.

73. Yamaguchi T, Omatsu N, Morimoto E, Nakashima H, Ueno K, Tanaka T, Satouchi K, Hirose F, Osumi T: **CGI-58 facilitates lipolysis on lipid droplets but is not involved in the vesiculation of lipid droplets caused by hormonal stimulation**. *J Lipid Res* 2007, **48**:1078-1089.

74. Evans CL, Potma EO, Puoris'haag M, Cote D, Lin CP, Xie XS: **Chemical imaging of tissue in vivo with video-rate coherent anti-Stokes Raman scattering microscopy**. *Proc Natl Acad Sci U S A* 2005, **102**:16807-16812.

75. •• Guo Y, Walther TC, Rao M, Stuurman N, Goshima G, Terayama K, Wong JS, Vale RD, Walter P, Farese RV: **Functional genomic screen reveals genes involved in lipid-droplet formation and utilization**. *Nature* 2008, advance online publication 13 April 2008. doi:10.1038/nature06928.
Large-scale gene knock-down in Drosophila cells identifies a couple of protein families with importance for formation and regulation of LD pools.