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The life cycle of lipid droplets

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Proteomic studies have revealed many potential functions of cytoplasmic lipid droplets, and recent activity has confirmed that these bona fide organelles are central not only for lipid storage and metabolism, but for development, immunity, and pathogenesis by several microbes. There has been a burst of recent activity on the assembly, maintenance and turnover of lipid droplets that reveals fresh insights. This review summarizes several novel findings in initiation of lipid droplet assembly, protein targeting, droplet fusion, and turnover of droplets through lipophagy.

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Cytoplasmic lipid droplets comprise a central hub for metabolism and cellular homeostasis. They are found in most if not all nucleated cells and even in several prokaryotes [1]. Tissues dedicated to their function exist in higher plants and animals, where they provide energy for the organism, and, at least in mammals, regulate appetite and energy metabolism at distant sites through release of adipokines [2]. Lipid droplets go far beyond providing fuel and regulating its use: the neutral lipids within their cores, triacylglycerols and steryl esters plus a variety of other lipids depending on tissue and cell type, are the source of hormones, secondary messengers, and plasmalogens [3,4]. They protect the cell from fatty acid-induced lipotoxicity [5]. The proteins in the surrounding phospholipid monolayer have roles not only in lipid metabolism but also in interorganellar communication [6••], development [7•], and immunity [8•,9]. Lipid droplets are likely linked to ER-mediated protein degradation [10•], and they are essential for assembly of viruses and for providing energy for their replication [9,11]. Awakening to the importance of this organelle, the effort to understand their structure, function, birth and death, have become areas of intensive research. There have been several outstanding reviews in the past 2–3 years on droplet cell biology (among them are [12,13•,14]). This mini-review will touch upon a few very recent findings and the controversies they address and raise.

Initiation of droplet assembly

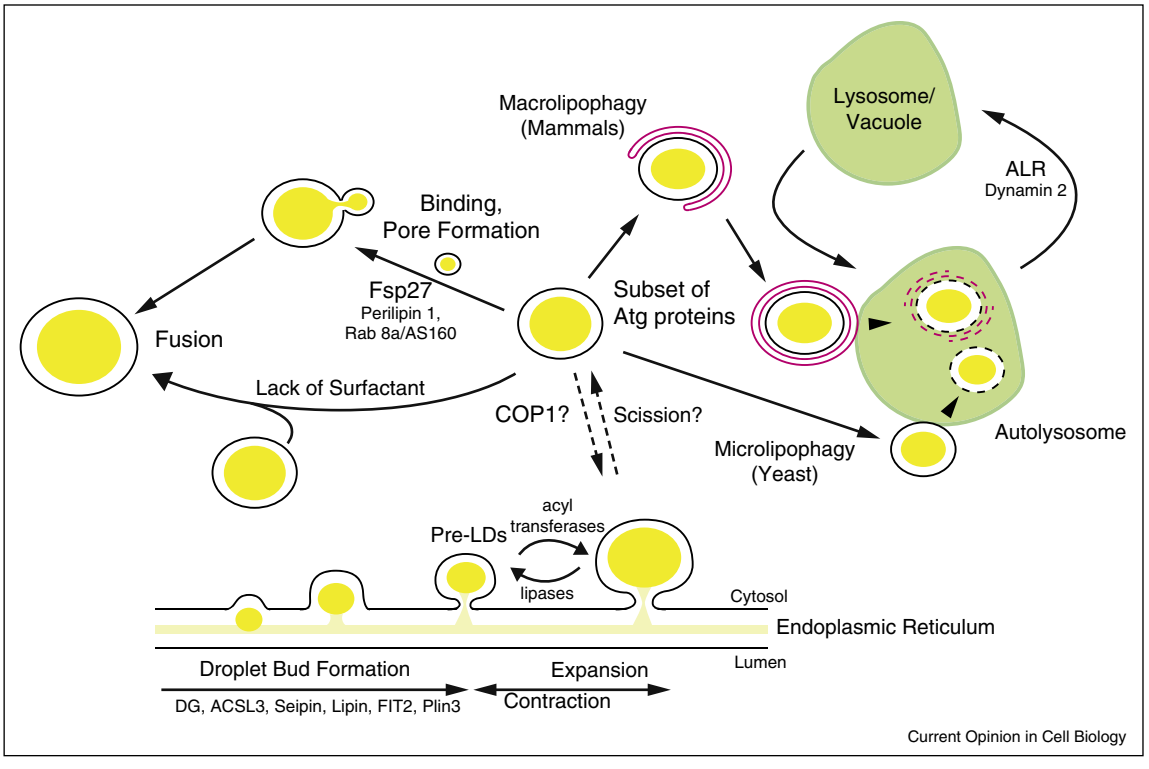
Lipid droplets originate in the endoplasmic reticulum; the terminal enzymes in the synthetic pathways that generate neutral lipids — mainly triacylglycerols (TG) and steryl esters (SE) – are localized there [15]. Since these acyltransferases have their active sites facing either the cytosolic or luminal side of the bilayer, neutral lipids can enter the bilayer from either direction [16,17]. Because model phospholipid bilayers can support a few mole percent in neutral lipids without sacrificing stability [18], it seems plausible that the ER membrane has a low level of neutral lipids freely diffusing within its bilayer. As saturation is reached, droplets will form. Recent application of emulsion chemistry to droplet formation is consistent with a spontaneous model for droplet formation, with the surrounding phospholipids (presumably derived from the ER outer leaflet) serving as the emulsifying agent [19•]. It is likely, however, that proteins play a role in development of the nascent droplet. For example, by embedding into the cytosolic side of the membrane they can stabilize or enhance the initial convex curvature to ensure that droplets bud from the cytosolic membrane leaflet and not into the ER lumen. Plin3, which binds to nascent droplets on the ER surface, is a good candidate for this function [20]. Proteins containing helical hairpins, such as GPAT4 or DGAT2, which traffic from the ER to droplets [21•,22•] may also contribute to vectorial budding. The initial generation of the bud may be promoted by FIT2, an ER protein that binds to triacylglycerols [23]; seipin, mutations in which cause severe lipodystrophy [24], may also be a player. Lipid droplet formation is delayed in the absence of seipin, leading to accumulation of neutral lipids in the ER and blebbing out into inappropriate sites such as the nucleus [25]. Curvature-producing lipids also may contribute to droplet formation. The outer leaflet of the ER membrane must deform, generating both a convex surface over the bud and a concave surface at the ER–bud interface. Diacylglycerol (DG), which would support convex curvature, has already been shown to promote droplet budding [20], and yeast lipin, which generates DG from phosphatidic acid (PA), is required to prevent a large accumulation of neutral lipid in the ER, even in the absence of TG synthesis [26]. This finding suggests that DG rather than PA (both of which promote shape change in the same direction) is more important for droplet assembly. In this regard, Fei *et al.*

have observed an increase in PA in the ER in seipin-deficient yeast [27], and our group has seen PA puncta in these cells (Han and Goodman, unpublished data), suggesting that PA accumulation caused by an absence of seipin may have an inhibitory effect on droplet formation. The salutary role of DG in droplet formation, therefore, may involve more than its membrane-curvature properties (Figure 1).

A long-standing question is whether droplet formation occurs at fixed sites in the ER, or whether these sites are random. In mammalian models of adipogenesis, droplets often first develop in the cell periphery, where the ER is rather sparse, and then migrate toward the nucleus [28]. To address whether sites on the ER are marked for droplet formation, Kassan *et al.* expressed in COS cells a 50-amino acid fusion peptide containing minimal ER and droplet targeting motifs. In starved cells this peptide formed puncta on the ER, even though droplets were not visible by normal staining methods. Upon addition of oleic acid, which rapidly becomes incorporated into neutral lipid, these puncta, termed pre-LDs, marked the sites of the first wave of droplet formation [29•]. Ultrastructural studies suggested the pre-LDs were tiny droplets of ~250 nm diameter in these starved cells which were apparently stable over time. Interestingly, the pre-LDs contain the acyl-CoA synthase ASCL3, but not perilipins 2 or 3, nor DGAT2 [29•]. Thus, a limited population of droplet precursors on the ER may always present to promote rapid lipid storage.

Another unresolved question is whether droplets, as they mature, separate from the ER. Early electron micrographic studies revealed the close association of the ER with droplets [30]. In yeast our group reported that 94% of droplets could not be resolved from the ER by fluorescence microscopy [31], as if they were attached. There is beauty in a permanent link between droplets and the ER, since bridges between the two organelles, which recently have been visualized [21•], could serve as a large buffer to changes in phospholipid mass on droplets during lipolysis or growth of droplets. Furthermore, protein trafficking between the ER and droplets would be facilitated by such bridges. However, an alternative mechanism involving COPI for removal of phospholipids from shrinking droplets has been proposed (see below), obviating the requirement of the ER as a sink. Moreover, GPAT4 only targets from the ER to a subset of droplets, suggesting a

Figure 1



The life cycle of a lipid droplet. *Legend*: Important events in the lipid droplet life cycle are shown. Recent advances include a pre-droplet organelle and associated ACSL3, a possible cycling of droplets associated and released from the ER mediated by COP1, the dual role of Fsp27 in lipolysis and droplet fusion, and two specific pathways of lipophagy, one common in mammals, the other in yeast. ALR, autophagic lysosome reformation. Although droplets may exist that are disconnected from the ER, the figure is not meant to imply that droplet expansion, fusion, or autophagy is limited to ER-connected or ER-dissociated droplets.