Autophagosomes and lipid droplets: no longer just chewing the fat

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Autophagosomes are organelles capable of sequestering and degrading diverse cytoplasmic cargo for nutritional and quality control purposes. Targeted are also lipid droplets (LDs), the cytoplasmic stores of neutral lipids. In this issue of *The EMBO Journal*, Shpilka *et al* (2015) show that the relationship between LDs and autophagosomes is far more intricate and that LDs regulate autophagosome biogenesis.

See also: T Shpilka *et al* (August 2015)

Autophagosomes (Mizushima *et al*, 2011) and lipid droplets (LDs) (Zechner *et al*, 2012) are intriguing cytoplasmic organelles that, based on their fundamental makeup differences, could not appear to be further apart. Autophagosomes are double-membrane (four leaflets) organelles with a lumen filled with cytosol or more complex cytoplasmic components. In contrast, LDs are delimited by a hemimembrane composed of just one leaflet/phospholipid monolayer and are filled with fat, comprised of neutral lipids, triglycerides/triacylglycerols (TGs), and cholesterol esters. Nonetheless, several recent studies (Singh *et al*, 2009; Velikkakath *et al*, 2012; Dupont *et al*, 2014; Rambold *et al*, 2015; Shpilka *et al*, 2015) have connected autophagosomes and LDs in a variety of ways (Fig 1).

One of the more widely accepted relationships is that autophagosomes can engulf LDs (Fig 1, pathway 1) in a process termed “lipophagy” (Singh *et al*, 2009) with the outcome being a lysosomal lipolysis and release of fatty acids for metabolic needs. The majority of studies have supported, albeit others have contested (Zechner *et al*, 2012) this view in the overall metabolic

mobilization of fat, lipolysis, and fatty acid β -oxidation. Recent work (Dupont *et al*, 2014; Rambold *et al*, 2015) has indicated that autophagosomes and LDs may have more surreptitious relationships than the simplistic view of “autophagosomes eat LDs”. One of these studies (Rambold *et al*, 2015) shows that during starvation, which is a classical inducer of autophagosome formation, the lipolysis is helped by autophagy but occurs not through “lipophagy”. Instead, lipolysis occurs through a classical pathway of mobilizing TGs through the adipose triacylglycerol lipase ATGL, which acts enzymatically on the surface of LDs (Zechner *et al*, 2012). Nevertheless, autophagy helps sustain this process by turning over other cytoplasmic components and, in the model proposed (Rambold *et al*, 2015), somehow replenishes TGs in LDs (Fig 1, pathway 2). The study by Shpilka *et al* (2015) goes much further than any of the earlier reports and flips the order in the relationship between LDs and autophagy: instead of being a substrate for lipophagy, LDs act as contributors to autophagosome biogenesis (Fig 1, pathway 3).

In their elegant study employing the power of yeast genetics, Shpilka *et al* (2015) demonstrate that enzymes essential for formation of LDs and responsible for TG (Dga1 and Lro1) and steryl-ester (Are1 and Are2) synthesis are also required for functional autophagy. This observation alone would be highly intriguing and raises many interesting questions. However, Shpilka *et al* (2015) go a step further to determine that the newly identified yeast TG lipase Ayr1 and the lipase Ldh1 (but not the well-established TG lipases Tgl3, Tgl4, Tgl5) are essential for autophagy. One plausible interpretation of these findings is that TGs

stored in LDs need to be mobilized in a specific fashion to enable autophagosome biogenesis. Since exogenously added fatty acids could not rescue autophagy in an LD-deficient strain of yeast, the requirement for LDs is not simply as a source of fatty acids and energy to support autophagosome biogenesis. Furthermore, Shpilka *et al* (2015) have shown that in addition to TG mobilization, steryl-ester mobilization through enzymes Tgl1, Yeh1, and Yeh2 is just as important. Finally, Ldh1, which has both TG and steryl-ester lipase/hydrolase activities, synergizes with the requirement for the TG lipase Ayr1, once again pointing to the necessity to mobilize all neutral lipid components of LDs to support autophagy. Of note, in contrast to the starvation-induced bulk autophagy, which requires large autophagosomes, the tighter, smaller autophagic organelles of the Cvt pathway did not show these requirements, indicating that it is an increased demand for lipids in the biogenesis of spacious autophagosomes that may be particularly sensitive to the ability to mobilize neutral lipids from LDs.

Finally, Shpilka *et al* (2015) show that ER–lipid droplet contact sites were important in these processes. Ice2 and Ldb16, which reside in the contact sites, couple TG utilization with lipid synthesis in the ER. They also control phospholipid synthesis and lipid droplet size determined by the phospholipid/neutral lipid ratios (Wang *et al*, 2014). Shpilka *et al* (2015) found that absence of either Ice2 or Ldb16 diminished starvation-induced autophagy. This resonates with the significance of the ER in autophagosome biogenesis, which is believed to be a major source for autophagosomal membranes. It furthermore underscores the role of LDs as an important

Figure 1. Intersections between lipid droplets (LD) and autophagosomes.

(1) Autophagosomes sequester LDs and digest TGs by acid lipolysis in autolysosomes (not depicted) to liberate fatty acids (FA) that are then oxidized in mitochondria. (2) Autophagosomes digest general cytoplasmic components to replenish triglycerides/triacylglycerol (TG) in lipid droplets thus sustaining LDs and surface lipolysis to liberate FAs that are burned in mitochondrial respiration. (3) Shpilka *et al* (2015) show that LDs and their constituents TGs as well as steryl-esters (SE) support autophagosome biogenesis possibly through lipid precursor and phospholipid (PL) flow to the growing autophagosomes. Solid arrow, proposed reverse flow through the endoplasmic reticulum (ER, light blue) via the LD–ER contact sites and ER–autophagosome contact sites; dashed arrow, potential exchange of lipids (phospholipids delimiting LDs or diacylglycerol liberated by lipases) via LD–autophagosome interactions.

contributor to autophagosome formation, since LDs emerge from the ER where TG synthesis occurs.

The study by Shpilka *et al* (2015) is not only elegant but also a very definitive demonstration of the precursor–product relationship between LDs and autophagosomes. In keeping with the result by Shpilka *et al* (2015) in yeast, Dupont *et al* (Dupont *et al*, 2014) have reported similar relationships in mammalian cells. In mammalian cells, the reserves of neutral lipids in LDs are needed for optimal autophagy of a variety of cargo (autophagic receptors, long-lived proteins, mitochondria, and invading microbes such as *M. tuberculosis*). Contacts

between LDs and autophagosomes have been observed (Yla-Anttila *et al*, 2009; Dupont *et al*, 2014), and the two organelles engage in kiss-and-run events (Dupont *et al*, 2014) that may allow direct exchange of the material. What might be the material that is exchanged in the transient kiss-and-run events (Dupont *et al*, 2014) or by the proposed reverse flow of lipids to the ER and then to autophagosomes (Shpilka *et al*, 2015)? A relevant type of molecules would be phospholipids or their precursors such as diacylglycerol (DAG) liberated from TGs by the action of lipases, as inferred by Shpilka *et al* (2015) in yeast. Indeed, a similar screen for TG-mobilizing enzymes in

mammalian cells has identified PNPLA5, a patatin-like phospholipase domain-containing protein and a member of the PNPLA1–PNPLA5 family (ATGL is PNPLA2), as co-localizing with the autophagic membrane factor ATG16L1 on LDs and playing a role in autophagy initiation/autophagosome elongation. This includes effects on conjugation of LC3 (mammalian Atg8) to phosphatidylethanolamine, a key event in autophagy best known as the conversion from LC3-I into its lipidated LC3-II form. In agreement with these observations, Shpilka *et al* (2015) found that during starvation, yeast Atg8 accumulated in its unlipidated form in the *tagΔ* mutant (*dga1Δ lro1Δ*) defective in TG synthesis. Intriguingly, it was the lipidated Atg8 form that accumulated in the *steΔ* and the *steΔtagΔ* strains defective in steryl-ester synthesis. Shpilka *et al* (2015) provide a tantalizing albeit tentative explanation for this as a possible influence of LDs on the lipidation and de-lipidation of Atg8, which apparently undergoes a rapid regulatory cycling between lipid-conjugated and deconjugated forms mediated by Atg4 (Nair *et al*, 2012).

These studies reveal a wealth of interactions between LDs and autophagosome biogenesis, with exciting possibilities for regulatory interactions as well as bulk lipid flow. According to Shpilka *et al* (2015), this lipid flow may go from LDs back to the ER from where they are mobilized into autophagosomal membranes, whereas others (Dupont *et al*, 2014) suggest that there may be an additional, direct kiss-and-run exchange between LDs and autophagosomes, whereby the LDs donate lipids to the outside membrane of the phagophore, possibly contributing to curvature through asymmetric loading from the phospholipid monolayer of the lipid droplet or by donating DAG as a curvature-inducing lipid.

It is enlightening to see the latest developments in the relationship between LDs and autophagosomes. A further twist in these interconnections is that autophagy factors have been directly (i.e. independently of the process of autophagy) implicated in controlling the size of LDs (Velikkakath *et al*, 2012). The latest studies also underscore the role of heterotypic inter-organelle contact sites (Yla-Anttila *et al*, 2009; Dupont *et al*, 2014; Rambold *et al*, 2015; Shpilka *et al*, 2015), a popular topic in cell biology in general. The attractiveness of these concepts is not in the controversy but

in being non-mutually exclusive, simply revealing multiple intersections between LDs and autophagy (Singh *et al*, 2009; Dupont *et al*, 2014; Shpilka *et al*, 2015) along with mitochondria (Rambold *et al*, 2015) and the ER. Given the role of lipid metabolism and autophagy in many disease-associated processes, understanding this particular set of relationships may be of potentially high payoff value.

References

- Dupont N, Chauhan S, Arko-Mensah J, Castillo EF, Masedunskas A, Weigert R, Robenek H, Proikas-Cezanne T, Deretic V (2014) Neutral lipid stores and lipase PNPLA5 contribute to autophagosome biogenesis. *Curr Biol* 24: 609–620
- Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 27: 107–132
- Nair U, Yen WL, Mari M, Cao Y, Xie Z, Baba M, Reggiori F, Klionsky DJ (2012) A role for Atg8-PE deconjugation in autophagosome biogenesis. *Autophagy* 8: 780–793
- Rambold AS, Cohen S, Lippincott-Schwartz J (2015) Fatty acid trafficking in starved cells: regulation by lipid droplet lipolysis, autophagy, and mitochondrial fusion dynamics. *Dev Cell* 32: 678–692
- Shpilka T, Welter E, Borovsky N, Amar N, Mari M, Reggiori F, Elazar Z (2015) Lipid droplets and their component triglycerides and steryl esters regulate autophagosome biogenesis. *EMBO J* 34: 2117–2131
- Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009) Autophagy regulates lipid metabolism. *Nature* 458: 1131–1135
- Velikkakath AK, Nishimura T, Oita E, Ishihara N, Mizushima N (2012) Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of LD. *Mol Biol Cell* 23: 896–909
- Wang CW, Miao YH, Chang YS (2014) Control of lipid droplet size in budding yeast requires the collaboration between Fld1 and Ldb16. *J Cell Sci* 127: 1214–1228
- Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL (2009) 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 5: 1180–1185
- Zechner R, Zimmermann R, Eichmann TO, Kohlwein SD, Haemmerle G, Lass A, Madeo F (2012) FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling. *Cell Metab* 15: 279–291