Lipid droplets at a glance

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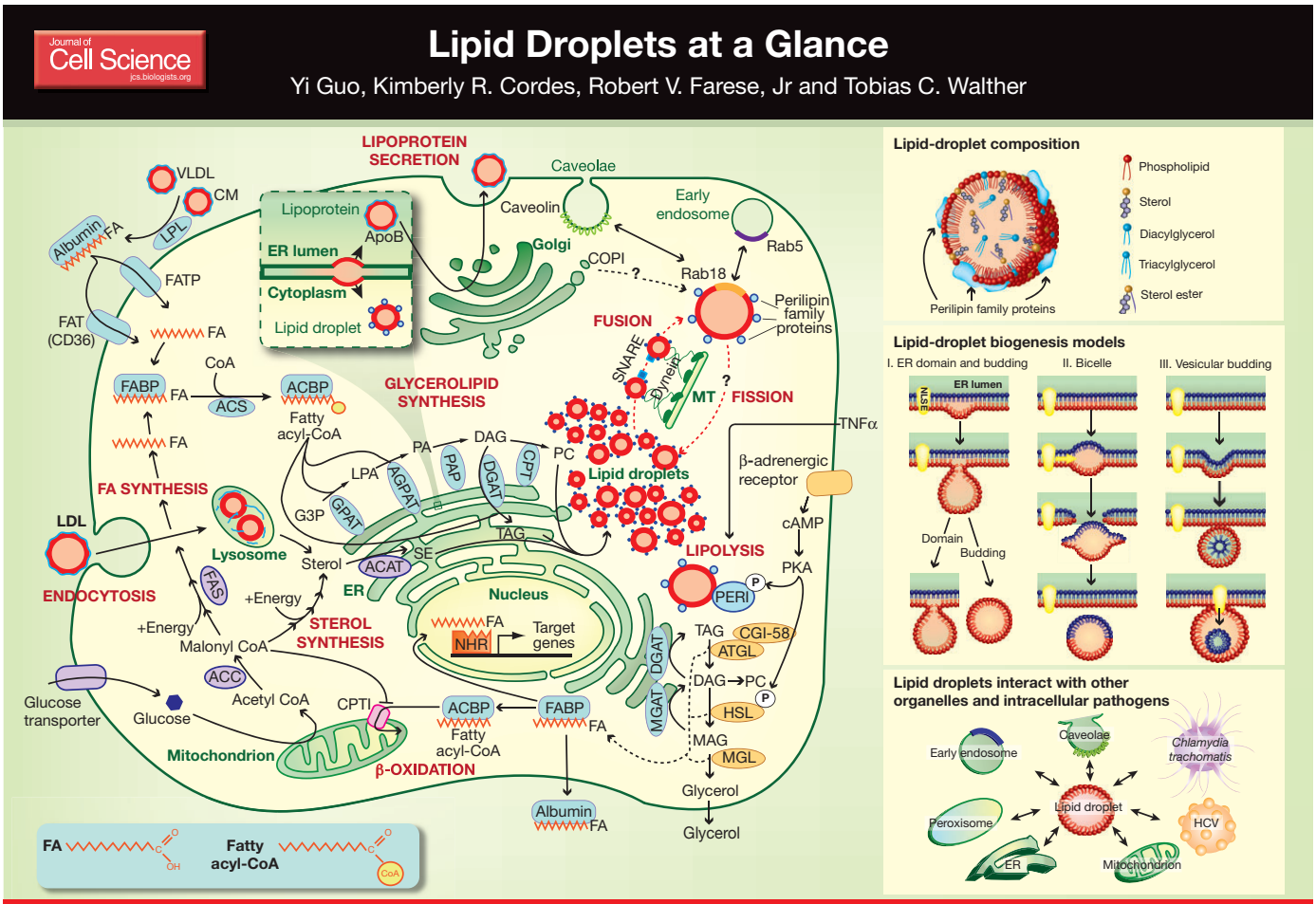
Journal of Cell Science 122, 749-752
Published by The Company of Biologists 2009
doi:10.1242/jcs.037630

Lipid droplets (LDs) are the major cellular organelles for the storage of neutral lipids. Excessive lipid storage in LDs is central to the pathogenesis of prevalent metabolic diseases, such as obesity, diabetes and atherosclerosis; LDs are, therefore, crucial in the development of these diseases. Long considered to be inert, LDs have recently attracted great interest as dynamic structures at the hub of lipid and energy metabolism. Major findings that highlight the diversity and dynamics of LDs include the identification of key proteins that are involved in LD biology, the discovery of differences in protein and lipid compositions of LDs in different cell types and physiological states, and the demonstration of interactions between LDs and other organelles [e.g. peroxisomes, endosomes, endoplasmic reticulum (ER), plasma membrane and mitochondria].

Despite the acceleration of progress in LD research and in determining the many links of LDs with prominent diseases, most fundamental questions are not yet resolved. How and where are LDs formed? How are proteins and lipids recruited to LDs? How do they interact with other organelles? How are their number, size and distribution regulated? In addition, new and unexpected connections with other cellular processes and pathologies are being found. Examples include the discovery that LDs play a role in histone storage in early *Drosophila melanogaster* development, and that LDs are required for efficient replication of intracellular pathogens, such as *Chlamydia trachomatis* and hepatitis C virus. Further research into LDs has also been sparked by the increased interest in biofuels; because these are mainly oils that are stored in LDs, efforts to manipulate systems to store more triglycerides in these organelles are also likely to benefit from a better understanding of the basic biology of fat storage. These and other questions pose many fascinating biological problems to be solved.



(See poster insert)

This article highlights emerging topics in LD biology and summarizes our current understanding of LD cellular functions. In the accompanying poster, the architecture of LDs is explained, and the biochemical pathways leading to fat storage are illustrated in a cellular context. In addition, we portray current models for LD formation and highlight the interactions of LDs with other organelles.

Lipid-droplet composition

LDs consist of an organic core comprising neutral lipids (mainly triacylglycerols and sterol esters) that is bounded by a monolayer of phospholipids (Bartz et al., 2007a). This structure provides a unique separation of the aqueous and organic phases of the cell. Several types of proteins decorate LDs (Bartz et al., 2007b; Brown, 2001), including structural proteins (e.g. proteins of the perilipin family) (Brasaemle, 2007), lipid-synthesis enzymes [e.g. acetyl coenzyme A (CoA) carboxylase, acyl-CoA synthetase and acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2)] (Kuerschner et al., 2008; Stone et al., 2009), lipases [e.g. adipose tissue triacylglycerol lipase (ATGL)] and membrane-trafficking proteins (e.g. Rab5, Rab18 and ARF1). Adding to the complexity, different LDs in a cell can contain different proteins (Ducharme and Bickel, 2008) and have different rates of acquiring triacylglycerol (Kuerschner et al., 2008). This suggests that cells contain distinct types of LDs with specialized functions.

Considering the composition of LDs, it is not well understood how proteins target to LDs and how this localization is regulated. Conceptually, proteins that contain transmembrane-spanning domains with hydrophilic domains on either side of a membrane bilayer cannot target to the monolayer surface of an LD; instead, there are at least two probable alternative mechanisms. First, proteins might use long membrane-embedded domains that enter and exit the membrane on the same side of the lipid monolayer. This mechanism was postulated for caveolins, which target to specialized domains of the plasma membrane and LDs (Martin and Parton, 2006), and for the lipid-synthesis enzyme DGAT2 (Kuerschner et al., 2008; Stone et al., 2006). Second, proteins might bind LD surfaces as peripheral membrane proteins by embedding an amphipathic helix. Examples include members of the perilipin family of proteins [e.g. perilipin, adipophilin, S3-12 and tail-interacting protein of 47 kDa (TIP47)] (Brasaemle, 2007). Whether a recently identified N-terminal hydrophobic sequence shared between several LD proteins [e.g. the putative methyltransferases AAM-B (methyltransferase-like protein 7A) and ALDI (associated with lipid droplet protein 1)] uses a similar targeting mechanism remains to be determined (Zehmer et al., 2008). Surprisingly, a variety of proteins have been detected in the hydrophobic core of LDs (Robenek et al., 2005), including the perilipin family of proteins. How such proteins are transported in and out of LDs, however, and whether they are natively folded in the LD are not clear.

Lipid-droplet formation

The life cycle of LDs begins when fatty acids that are carried extracellularly by albumin and lipoproteins enter cells. Fatty acids are released from triacylglycerols in lipoproteins by lipoprotein lipase, and enter cells by passive diffusion facilitated by fatty-acid transport proteins or fatty-acid translocase (Ehehalt et al., 2006; Schaffer and Lodish, 1994). Fatty acids can also be synthesized de novo from carbohydrates in many cell types.

Next, fatty acids enter a bioactive pool through conjugation to CoA, forming fatty acyl-CoA, in an energy-requiring reaction. Fatty acyl-CoA is used by glycerolipid-synthesis enzymes (glycerol-3-phosphate acyltransferase and *sn*-1-acylglycerol-3-phosphate acyltransferase) in the ER to ultimately generate diacylglycerols. Diacylglycerols are either converted to neutral lipids (triacylglycerols) by DGAT enzymes or enter phospholipid-synthesis pathways. How the flux between these pathways is regulated is unknown.

In contrast to fatty acids, sterols are primarily taken up into cells through endocytosis and lysosomal degradation of lipoproteins. Most cells can also synthesize sterols. Excess sterols are converted to sterol esters through conjugation with fatty acyl-CoA in a reaction that is catalyzed by sterol-*O*-acyltransferases (e.g. acyl-CoA:cholesterol acyltransferase) in the ER.

Thus, neutral lipids that are found in LD cores are synthesized in the ER. How these lipids accumulate and form LDs is mostly unknown. The canonical model posits that neutral lipids form a lens of oil in the ER bilayer that subsequently 'buds' from the membrane (the ER-budding model), taking with it phospholipids from the cytosolic leaflet. Although the model has substantial support, this process has not been observed directly. In a variant of this model, the ER-domain model, LDs remain connected to the ER and are lipid-containing protrusions of the ER membrane, forming a specialized ER domain.

Alternative models for LD formation have been proposed. In the bicelle model (Ploegh, 2007), neutral lipids accumulate between the leaflets of the ER membrane but, instead of budding, nascent LDs are excised from the membrane, taking with them phospholipids from both the cytosolic and luminal leaflets. This model was suggested to explain how large unfolded proteins or viruses might escape from the ER lumen into the cytosol. In the vesicular-budding model (Walther and Farese, 2008), small bilayer vesicles that remain tethered to the ER membrane are used as a platform for making LDs. Newly synthesized neutral lipids are pumped into the vesicle bilayer and fill the intermembrane space, eventually squeezing the vesicular lumen so that it becomes a small inclusion inside the LDs. Clues that help to decipher how LDs are formed might be provided by studying seipin, an ER protein that is integral to LD formation (Fei et al., 2008; Szymanski et al., 2007).

In lipoprotein-producing cells, such as intestinal enterocytes or hepatocytes, neutral lipids can also be directed from the ER bilayer into the ER lumen to associate with apolipoprotein B for secretion (Fujimoto et al., 2008). How the amount of neutral lipids entering the storage versus the secretion pathway is determined is mostly unknown.

Lipid-droplet growth

The size of LDs varies tremendously, with diameters ranging from as small as 20-40 nm (Stobart et al., 1986) to 100 μm (in white adipocytes), and, clearly, LDs can grow in size. How do LDs grow so dramatically?

One possibility is that LDs, like balloons, expand as single organelles. If LDs remain attached to the ER, proteins and newly synthesized lipids could diffuse laterally to the LDs. If LDs are detached from the ER, these proteins and lipids must be

transported to the LDs, perhaps via vesicular transport. This would pose a problem of topology, as a vesicular bilayer membrane would have to fuse with a monolayer surface at the LD. Alternatively, neutral lipids in the core could be produced locally by enzymes, such as DGATs, that are targeted to the LD surface. In either scenario, the increase in volume of neutral lipids would need to be matched by a corresponding increase of phospholipids at the surface. In agreement with this notion, a key enzyme of phospholipid synthesis, CTP:phosphocholine cytidylyltransferase (CCT), is localized to LD surfaces during their growth (Guo et al., 2008).

Fusion of smaller LDs to form larger LDs is also likely to contribute to LD growth (e.g. Guo et al., 2008), and models that implicate SNARE proteins and motor proteins in LD fusion have been proposed (Boström et al., 2007; Olofsson et al., 2008). A fusion mechanism would alleviate the requirement for phospholipid synthesis during the growth of LDs, because the surface:volume ratio decreases with fusion.

Lipid-droplet mobilization

Neutral lipids in LDs are mobilized by lipases to provide metabolic energy (through the oxidation of fatty acids) and lipids for membrane synthesis (Brasaemle, 2007; Ducharme and Bickel, 2008; Zechner et al., 2005). In adipocytes, this lipolysis is triggered by hormonal, nutritional or inflammatory (such as tumor necrosis factor-α) signals. For example, catecholamines bind to β-adrenergic G-protein-coupled receptors at the plasma membrane and activate adenylyl cyclase, generating cAMP. This second messenger activates protein kinase A, which directly phosphorylates perilipin and hormone-sensitive lipase (HSL). Hormone-stimulated phosphorylation of perilipin releases CGI-58 from the CGI-58–perilipin complex, freeing CGI-58 to activate ATGL (Yamaguchi et al., 2007), and recruits phosphorylated HSL to the LD surfaces. Together, ATGL and HSL hydrolyze triacylglycerols to fatty acids and monoacylglycerols, which are further catabolized to yield glycerol and fatty acids. The partially hydrolyzed intermediates (diacylglycerols and monoacylglycerols) can also be used to synthesize phospholipids. Mutations in CGI-58 impair lipolysis (Igal and Coleman, 1998; Lass et al., 2006), implicating it as a key cofactor. CGI-58 also has lysophosphatidic-acid-specific acyltransferase activity (Ghosh et al., 2008) of unclear significance. The fatty acids that are liberated from lipolysis may be activated to acyl-CoA and transported to mitochondria for β-oxidation to provide cellular ATP, may enter the nucleus, where they act as ligands for nuclear hormone receptors and regulate gene transcription, or may be released from the cells to provide fuel or signaling molecules for other cells or tissues.

During lipolysis, LDs might undergo fission, which would dramatically increase the surface area of LDs and enable lipases to better access the neutral-lipid cores. Fission of LDs has been observed in adipocytes after massive lipolytic stimulation (Marcinkiewicz et al., 2006). As the surface area of LDs expands with fission, more surface phospholipids would be required. This requirement might explain why a reduction in phospholipid synthesis through the knockdown of CCT leads to a defect in lipolysis (Guo et al., 2008).

Lipid droplets interact with other cellular organelles

There is increasing evidence that LDs dynamically interact with other cellular organelles. In particular, LDs are often found in close approximation with the ER, mitochondria, endosomes, peroxisomes and the plasma membrane (Goodman, 2008; Murphy et al., 2008). The functions of these interactions are still largely unknown. These organelle associations might facilitate the exchange of lipids, either for anabolic growth of LDs or for their catabolic breakdown. Alternatively, LDs might provide a means of transporting lipids between organelles within the cell, much like lipoproteins transport lipids between tissues via the blood. Some of the interactions between LDs and other organelles might be mediated and regulated by Rab GTPases, which have been found on LDs (Liu et al., 2008).

Unexpected functions for lipid droplets

Besides storing key lipids, recent studies suggest that LDs might have other functions in cellular physiology or pathology. For example, LDs might act as the protective reservoir for unfolded proteins or other compounds (that are sequestered in the organic phase) to prevent harmful interactions with other cellular components (Ohsaki et al., 2006; Welte, 2007).

The intracellular pathogen *C. trachomatis* induces the accumulation of LDs around the bacteria-replication vacuole and appears to use LD components for replication (Cacchiaro et al., 2008; Kumar et al., 2006). Similarly, the hepatitis C virus appears to use LDs as the platform for viral assembly, and blocking the association of the hepatitis C core protein with LDs impairs viral replication (Miyanari et al., 2007). A better understanding of these LD-pathogen interactions might provide new avenues for therapeutic interventions.

Perspectives

The past several years have witnessed a boom in research on the cellular biology of LDs. However, almost all mechanistic details concerning LDs are unclear. How exactly are LDs formed? How are proteins targeted to LDs? How is fusion and fission of LDs regulated? Do LDs traffic between organelles? During lipolysis, how do lipases access neutral lipids in the LD core? Answers to these exciting questions will reveal much about the basic mechanisms of cellular lipid and energy metabolism, and might provide useful knowledge for therapeutic and biotechnological applications.

We thank Peter Walter, Neil Sheehy, and members of the Farese and Walther laboratories for helpful discussion and comments on the manuscript; Daryl Jones for manuscript preparation; and Gary Howard and Stephen Ordway for editorial assistance. This work was supported by NIH grant R21-DK78254 (to R.V.F., Jr), a David and Mary Phillips postdoctoral fellowship award (to Y.G.), the International Human Frontier Science Program Organization (T.C.W.), the J. David Gladstone Institutes (Y.G, K.R.C. and R.V.F., Jr), and the Max-Planck Society (T.C.W.). Deposited in PMC for release after 12 months.

References

Bartz, R., Li, W. H., Venables, B., Zehmer, J. K., Roth, M. R., Welti, R., Anderson, R. G., Liu, P. and Chapman, K. D. (2007a). Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic. *J. Lipid Res.* **48**, 837-847.

Bartz, R., Zehmer, J. K., Zhu, M., Chen, Y., Serrero, G., Zhao, Y. and Liu, P. (2007b). Dynamic activity of lipid droplets: protein phosphorylation and GTP-mediated protein translocation. *J. Proteome Res.* **6**, 3256-3265.

Boström, P., Andersson, L., Rutberg, M., Perman, J., Lidberg, U., Johansson, B., Fernandez-Rodriguez, J., Ericson, J., Nilsson, T., Borén, J. et al. (2007). SNARE proteins mediate fusion between cytosolic lipid droplets and are implicated in insulin sensitivity. *Nat. Cell Biol.* **9**, 1286-1293.

Brasaemle, D. L. (2007). The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. *J. Lipid Res.* **48**, 2547-2559.

Brown, D. A. (2001). Lipid droplets: proteins floating on a pool of fat. *Curr. Biol.* **11**, R446-R449.
Cacchiaro, J. L., Kumar, Y., Fischer, E. R., Hackstadt, T. and Valdivia, R. H. (2008). Cytoplasmic lipid droplets are translocated into the lumen of the Chlamydia trachomatis parasitophorous vacuole. *Proc. Natl. Acad. Sci. USA* **105**, 9379-9384.
Ducharme, N. and Bickel, P. (2008). Lipid droplets in lipogenesis and lipolysis. *Endocrinology* **149**, 942-949.
Ehehalt, R., Füllekrug, J., Pohl, J., Ring, A., Herrmann, T. and Stremmel, W. (2006). Translocation of long chain fatty acids across the plasma membrane-lipid rafts and fatty acid transport proteins. *Mol. Cell Biochem.* **284**, 135-140.
Fei, W., Shui, G., Gaeta, B., Du, X., Kuerschner, L., Li, P., Brown, A., Wenk, M., Parton, R. and Yang, H. (2008). Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast. *J. Cell Biol.* **180**, 473-482.
Fujimoto, T., Ohsaki, Y., Cheng, J., Suzuki, M. and Shinohara, Y. (2008). Lipid droplets: a classic organelle with new outfits. *Histochem. Cell Biol.* **130**, 263-279.
Ghosh, A. K., Ramakrishnan, G., Chandramohan, C. and Rajasekharan, R. (2008). CGI-58, the causative gene for Chanarin-Dorfman syndrome, mediates acylation of lysophosphatidic acid. *J. Biol. Chem.* **283**, 24525-24533.
Goodman, J. M. (2008). The gregarious lipid droplet. *J. Biol. Chem.* **283**, 28005-28009.
Guo, Y., Walther, T. C., Rao, M., Stuurman, N., Goshima, G., Terayama, K., Wong, J. S., Vale, R. D., Walter, P. and Farese, R. V., Jr (2008). Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* **453**, 657-661.
Igal, R. A. and Coleman, R. A. (1998). Neutral lipid storage disease: a genetic disorder with abnormalities in the regulation of phospholipid metabolism. *J. Lipid Res.* **39**, 31-43.
Kuerschner, L., Moessinger, C. and Thiele, C. (2008). Imaging of lipid biosynthesis: how a neutral lipid enters lipid droplets. *Traffic* **9**, 338-352.
Kumar, Y., Cocchiaro, J. and Valdivia, R. H. (2006). The obligate intracellular pathogen chlamydia trachomatis targets host lipid droplets. *Curr. Biol.* **16**, 1646-1651.
Lass, A., Zimmermann, R., Haemmerle, G., Riederer, M., Schoiswohl, G., Schweiger, M., Kienesberger, P., Strauss, J. G., Gorkiewicz, G. and Zechner, R. (2006). Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. *Cell Metab.* **3**, 309-319.
Liu, P., Bartz, R., Zehmer, J., Ying, Y. and Anderson, R. (2008). Rab-regulated membrane traffic between adiposomes and multiple endomembrane systems. *Methods Enzymol.* **439**, 327-337.
Marcinkiewicz, A., Gauthier, D., Garcia, A. and Brasaemle, D. (2006). The phosphorylation of serine 492 of perilipin a directs lipid droplet fragmentation and dispersion. *J. Biol. Chem.* **281**, 11901-11909.
Martin, S. and Parton, R. G. (2006). Lipid droplets: a unified view of a dynamic organelle. *Nat. Rev. Mol. Cell. Biol.* **7**, 373-378.
Miyanari, Y., Atsuzawa, K., Usuda, N., Watashi, K., Hishiki, T., Zayas, M., Bartenschlager, R., Wakita, T., Hijikata, M. and Shimotohno, K. (2007). The lipid droplet is an important organelle for hepatitis C virus production. *Nat. Cell Biol.* **9**, 1089-1097.
Murphy, S., Martin, S. and Parton, R. G. (2008). Lipid droplet-organelle interactions; sharing the fats. *Biochim. Biophys. Acta* [Epub ahead of print] doi:10.1016/j.bbalip.2008.07.004.
Ohsaki, Y., Cheng, J., Fujita, A., Tokumoto, T. and Fujimoto, T. (2006). Cytoplasmic lipid droplets are sites of convergence of proteasomal and autophagic degradation of apolipoprotein B. *Mol. Biol. Cell* **17**, 2674-2683.
Olofsson, S. O., Boström, P., Andersson, L., Rutberg, M., Levin, M., Perman, J. and Borén, J. (2008). Triglyceride containing lipid droplets and lipid droplet-associated proteins. *Curr. Opin. Lipidol.* **19**, 441-447.
Ploegh, H. (2007). A lipid-based model for the creation of an escape hatch from the endoplasmic reticulum. *Nature* **448**, 435-438.
Robenek, H., Robenek, M. and Troyer, D. (2005). PAT family proteins pervade lipid droplet cores. *J. Lipid Res.* **46**, 1331-1338.
Schaffer, J. E. and Lodish, H. F. (1994). Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* **79**, 427-436.
Stobart, A. K., Stymne, S. and Höglund, S. (1986). Safflower microsomes catalyse oil accumulation *in vitro*: a model system. *Planta* **169**, 33-37.
Stone, S. J., Levin, M. C. and Farese, R. V., Jr (2006). Membrane topology and identification of key functional amino acid residues of murine acyl-CoA:diacylglycerol acyltransferase-2. *J. Biol. Chem.* **281**, 40273-40282.
Stone, S. S., Levin, M. C., Zhou, P., Han, J., Walther, T. C. and Farese, R. V., Jr (2009). The endoplasmic reticulum enzyme, DGAT2, is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria. *J. Biol. Chem.* **284**, 5352-5361.
Szymanski, K., Binns, D., Bartz, R., Grishin, N., Li, W., Agarwal, A., Garg, A., Anderson, R. and Goodman, J. (2007). The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proc. Natl. Acad. Sci. USA* **104**, 20890-20895.
Walther, T. C. and Farese, R. V., Jr (2008). The life of lipid droplets. *Biochim. Biophys. Acta* [Epub ahead of print] doi:10.1016/j.bbalip.2008.10.009.
Welte, M. A. (2007). Proteins under new management: lipid droplets deliver. *Trends Cell Biol.* **17**, 363-369.
Yamaguchi, T., Omatsu, N., Morimoto, E., Nakashima, H., Ueno, K., Tanaka, T., Satouchi, K., Hirose, F. and Osumi, T. (2007). CGI-58 facilitates lipolysis on lipid droplets but is not involved in the vesiculation of lipid droplets caused by hormonal stimulation. *J. Lipid Res.* **48**, 1078-1089.
Zechner, R., Strauss, J. G., Haemmerle, G., Lass, A. and Zimmermann, R. (2005). Lipolysis: pathway under construction. *Curr. Opin. Lipidol.* **16**, 333-340.
Zehmer, J. K., Bartz, R., Liu, P. and Anderson, R. G. (2008). Identification of a novel N-terminal hydrophobic sequence that targets proteins to lipid droplets. *J. Cell Sci.* **121**, 1852-1860.

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