The topology of the triacylglycerol synthesizing enzyme Lro1 indicates that neutral lipids can be produced within the luminal compartment of the endoplasmatic reticulum

Implications for the biogenesis of lipid droplets

Vineet Choudhary, Nicolas Jacquier and Roger Schneiter*
Department of Biology; Division of Biochemistry; University of Fribourg; Fribourg, Switzerland

Eukaryotes store metabolic energy in form of neutral lipids, which are deposited within a dedicated organelle, termed lipid droplet (LD). While neutral lipids are synthesized by ER localized integral membrane proteins, the fate of these lipids after their synthesis and the mechanism resulting in their accumulation in LDs are not well understood. We have recently shown that LDs are functionally connected to the ER membrane allowing for a bidirectional and energy-independent transport of integral membrane proteins and possibly lipids between the two compartments during lipogenesis or lipolysis. To further characterize the nature of this connection, we investigated the topology of triacylglycerol (TAG) formation. Here we show that the active site residues of the TAG biosynthetic enzyme in yeast, Lro1, a homolog of the lecithin cholesterol acyltransferase (LCAT)-related proteins, are located within the ER luminal domain of the enzyme, suggesting that TAG formed by Lro1 is initially present in the ER luminal leaflets of the ER membrane. The topology of TAG formed by Lro1 thus contrasts that of the second TAG biosynthetic enzyme, Dga1, which has a cytosolic acyl-CoA binding domain and thus is likely to catalyze TAG formation in the cytosolic leaflet of the ER membrane. Since TAG formed by either Dga1 or Lro1 can be efficiently packed into LDs we conclude that neutral lipids from both the cytosolic as well as the luminal leaflet of the ER membrane can be concentrated and packed into LDs.

In eukaryotic cells an excess of free fatty acids and sterols (cholesterol in humans, ergosterol in yeast) is converted to neutral lipids, notably triacylglycerols (TAG) and steryl esters (STE), which are deposited into a dedicated storage compartment, called lipid droplet (LD). LDs are composed of a core of neutral lipids that is surrounded by an unusual phospholipid monolayer onto which LD-localized proteins appear to specifically associate. Some of these LD-localized proteins appear to fulfill structural functions such as the perilipins in mammals and oleosins in plants while others have well established functions in lipid synthesis or breakdown of neutral lipids, including lipases and acyltransferases.1-5

The prevailing hypothesis of LD biogenesis postulates that neutral lipids accumulate within the hydrophobic core of the ER membrane, where the lipid droplet grows and finally buds into the cytoplasm, surrounded by a membrane monolayer that is derived from the former cytoplasmic leaflet of the ER membrane.6 Despite the apparently wide acceptance of this model, evidence to support this hypothesis is rare and controversial.7,8 Inclusion of neutral lipids within the ER bilayer, or LDs budding from the ER into the cytosol, for example, have never been unequivocally observed.

LDs in Saccharomyces cerevisiae serve the same function and are structurally related to those of mammalian cells and plants.9 Neutral lipids synthesis and thus LD formation in yeast is under control

Key words: lipid droplets, endoplasmatic reticulum, triacylglycerol, steryl esters, lipoproteins, organelle biogenesis, Saccharomyces cerevisiae

Submitted: 08/22/11
Accepted: 08/22/11
DOI: 10.4161/cib.4.6.17830

*Correspondence to: Roger Schneiter; Email: roger.schneiter@unifr.ch

Addendum to: Jacquier N, Choudhary V, Mari M, Touilmy A, Reggiori F, Schneiter R. Lipid droplets are functionally connected to the endoplasmic reticulum in Saccharomyces cerevisiae. J Cell Sci 2011; 124:3424–37; DOI:10.1242/jcs.076836; PMID:21693588.

Addendum to: Jacquier N, Choudhary V, Mari M, Touilmy A, Reggiori F, Schneiter R. Lipid droplets are functionally connected to the endoplasmic reticulum in Saccharomyces cerevisiae. J Cell Sci 2011; 124:3424–37; DOI:10.1242/jcs.076836; PMID:21693588.

www.landesbioscience.com
Communicative & Integrative Biology 781

©2011 Landes Bioscience
of four enzymes, two of which produce TAG and two make STE. The acyl-CoA:sterol acyltransferases (ACATs), Are1 or Are2, are polytopic ER membrane proteins that synthesize STE. TAG, on the other hand, can be produced either by Lro1 or Dga1. Dga1 catalyzes the acyl-CoA-dependent synthesis of TAG from diacylglycerol. Dga1 has a hairpin like topology with both its N- and C-termini facing the cytosol, and a conserved lipid binding region which maps to the first transmembrane domain, suggesting that Dga1 accepts cytosolic acyl-CoA and synthesizes TAG from membrane-embedded substrate, diacylglycerol, into the cytosolic leaflet of the ER membrane.

The second TAG biosynthetic enzyme, Lro1, has homology to lecithin cholesterol acyltransferase (LCAT)-related proteins and catalyzes TAG formation through the CoA-independent transesterification of a fatty acid from phospholipids to diacylglycerol. Synthesis and storage of neutral lipids is dispensable for the viability of S. cerevisiae because a dga1Δ lro1Δ are1Δ are2Δ quadruple mutant is viable and lacks detectable LDs.

By using a genetic system that allows for the controlled induction of neutral lipid formation and hence LD biogenesis, we have recently reported that in cells which have no LDs, otherwise LD localized proteins are homogenously distributed within the ER membrane. Upon induction of LD formation, these proteins begin to concentrate over discrete regions within the ER membrane where neutral lipids appear to accumulate, indicating that nascent LDs are indeed formed at the ER membrane. Photobleaching (FRAP) experiments combined with immunoelectron microscopy, revealed that integral membrane proteins, which are biosynthetically inserted into the ER membrane, are transported from the ER to LDs in a pathway that is independent of vesicle formation and fusion and that is not blocked upon energy-depletion or at low temperature, suggesting a passive, possible diffusion-driven mechanism for the biosynthetic transport of integral membrane proteins from the ER to LDs. These data thus indicate that LDs are functionally connected to the ER membrane, allowing the bidirectional transport of integral membrane proteins and possibly lipids between the two compartments as required during lipogenesis or lipolysis. At a morphological level, this connection between the ER and LDs may be established via a membrane bridge that allows the diffusion of integral membrane proteins and lipids between the two compartments, alternatively, LDs may not form independent cytosolic structures as is frequently postulated, but they may either be enwrapped by the ER membrane and even form a domain within the ER that is enriched in neutral lipids and proteins that associate with these neutral lipid-rich domains, as has been proposed based on freeze fracture data, which reported membrane continuity between the ER and the surface of LDs. To investigate the relation between neutral lipid formation and LD structure in more detail we were interested in determining the topology of the TAG biosynthetic enzyme, Lro1. Here we show that the active site residues of Lro1 are located within the ER luminal domain of the enzyme suggesting that TAG formed by the action of Lro1 is initially present within the luminal leaflet of the ER membrane. The two TAG biosynthetic enzymes, Dga1 and Lro1 thus differ with respect to their topological orientation and precise site at which TAG is formed, Dga1 into the cytosolic leaflet of the ER membrane and Lro1 into the ER luminal leaflet of the ER membrane.

Analysis of the number and position of potential transmembrane domains of Lro1 indicates that the enzyme contains one predicted transmembrane domain located between amino acids 80 and 100. To determine whether Lro1 is indeed an integral membrane protein and to examine the membrane topology of the enzyme, functional N- and C-terminally epitope-tagged versions of Lro1 were generated. Functionality of these tagged versions of the enzyme was assessed by examining TAG formation in cells that express the tagged enzyme as the only source to catalyze TAG formation (data not shown). Subcellular fractionation revealed that Lro1 is enriched in a 13k microsomal fraction and thus that the protein is membrane associated, consistent with previous characterizations of its enzymatic activity. Extraction of microsomal membranes with salt or exposure to alkaline pH did not render the enzyme soluble (Fig. 1B). Lro1 was solubilized upon detergent treatment of microsomes only, indicating that Lro1 is an integral membrane protein, as is Wbp1, a component of the oligosaccharyl transferase complex, used as a control (Fig. 1B). Protease protection experiments in the presence and absence of detergent revealed that the N-terminus of Lro1 is protease accessible whereas the C-terminus is protected from protease digestion, indicating Lro1 has an odd number of transmembrane domains (Fig. 1C). Lro1 contains 9 potential N-linked glycosylation sites, all of which are located C-terminal to the transmembrane domain. At least one of these sites is indeed glycosylated as revealed by treatment with endoglycosidase H, indicating an ER luminal orientation of the C-terminal part of Lro1 (Fig. 1D). Lro1 belongs to the superfamly of hydrolytic enzymes having an α/β-hydrolase-fold with a catalytic mechanism that is similar to that of serine proteases and lipases, with a catalytic triad consisting of a Ser, His and an acidic amino acid. Based on structural and functional homology with several lipases, the active site residues of Lro1 were predicted and they correspond to S205, D567 and H617 of the yeast enzyme. Based on these structural considerations and the fact that the C-terminal domain of Lro1 must be localized in the ER lumen, where N-glycosylation occurs, we propose a model for the topology of Lro1 with a single transmembrane domain and a large ER luminal domain containing the active site residues within an α/β hydrolase fold (Fig. 1E). Consistent with our finding that the active site of the enzyme is localized in the ER lumen, Lro1 can be converted into a soluble enzymatically active and secreted protein by removal of the transmembrane region.

It is interesting to note that the biogenesis of LDs from ER luminal produced TAG, as is indicated by the membrane topology of Lro1, is topologically equivalent to the biogenesis of lipoprotein particles, a well defined pathway for the packaging and secretion of neutral lipid containing globules from the liver. Similar to LDs, very low-density lipoproteins (VLDLs) are also composed of a
hydrophobic core of TAG and STE that is surrounded by a phospholipid monolayer and one apolipoprotein (apo) B protein. Assembly and maturation of VLDLs is generally believed to include two lipidation steps, the first of which occurs in the ER lumen and involves the addition of small amounts of neutral lipids to apoB-100 during its translocation across the ER membrane to form a partially lipidated, pre-VLDL particle.23,24 The topology of this first lipidation step is assigned to the ER lumen and requires an ER resident protein, microsomal triglyceride transfer protein (MTP), indicating that in this system, TAG is available from within the ER lumen.25

Recent molecular dynamics simulations indicate that TAG sequesters within a model bilayer membrane and spontaneously aggregates within the center of the bilayer. These TAG aggregates appear to remain stable on the microsecond scale, giving the bilayer a blister-like appearance.26 In this model, TAG could probably enter the neutral lipid aggregate from either side of the bilayer, which would effectively neutralize any difference in the topology of its original synthesis.

Acknowledgments

This work was supported by the Novartis Foundation (08A09) and the Swiss National Science Foundation (PP00A-110450 and 3100-120650).

References

1. Olofsson SO, Bostrom P, Andersson L, Rutherford M, Pernman J, Boren J. Lipid droplets as dynamic organelles connecting storage and efflux of lipids. Biochim Biophys Acta 2009; 1791:448-58.
2. Goodman JM. The gregarious lipid droplet. J Biol Chem 2008; 283:28005-9; PMID:18611863; DOI:10.1074/jbc.R800042200.
3. Ducharme NA, Bickel PE. Lipid droplets in lipogenesis and lipolysis. Endocrinology 2008; 149:942-9; PMID:18202123; DOI:10.1210/ed.2007-1713.
4. Brasamle DL. The perilipin family of structural lipid droplet proteins: Stabilization of lipid droplets and control of lipolysis. J Lipid Res 2007; 48:2547-59; PMID:17878492; DOI:10.1194/jlr.R700014-JLR200.
5. Lass A, Zimmermann R, Oberer M, Zechner R. Lipolysis—a highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. Prog Lipid Res 2011; 50:34-27; PMID:21887632; DOI:10.1016/j.plipres.2010.10.004.
6. Murphy DJ, Vance J. Mechanisms of lipolysis—formation. Trends Biochem Sci 1999; 24:109-15; PMID:10203758; DOI:10.1016/S0969-8004(98)01349-8.
7. Murphy DJ. The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog Lipid Res 2001; 40:325-438; PMID:11470406; DOI:10.1016/S0163-7827(01)00013-3.
8. Fujimoto T, Ohkaki Y, Cheng J, Suzuki M, Shinohara Y. Lipid droplets: a classic organelle with new outfits. Histochem Cell Biol 2008; 130:263-79; PMID:18546515; DOI:10.1007/s00418-008-0449-0.
9. Leber R, Zinser E, Zellnig G, Palma F, Daum G. Characterization of lipid particles of the yeast, Saccharomyces cerevisiae. Yeast 1994; 10:1421-8; PMID:7871881; DOI:10.1002/yea.320101105.
10. Crabtree T, Athenaestad K, Daum G. Synthesis, storage and degradation of neutral lipids in yeast. Biochim Biophys Acta 2007; 1771:299-309.
11. Oelkers P, Cromley D, Padamsee M, Billheimer JT, Starley SL. The DgA1 gene determines a second triglyceride synthetic pathway in yeast. J Biol Chem 2002; 277:8877-81; PMID:11751875; DOI:10.1074/jbc.M111646200.
12. Sørgaard K, Lauga G. Synthesis of triacylglycerols by the acyl-coenzyme A:diacyl-glycerol acyltransferase Dga1p in lipid particles of the yeast Saccharomyces cerevisiae. J Bacteriol 2002; 184:519-24; PMID:11751830; DOI:10.1128/JB.184.2.519-24.2002.
13. Stone SJ, Levin MC, Farese RVJ. Membrane topology and identification of key functional amino acid residues of murine acyl-CoA:diacylglycerol acyltransferase-2. J Biol Chem 2006; 281:40273-82; PMID:17035227; DOI:10.1074/jbc.M600796200.
14. Liu Q, Siloto RM, Snyder CL, Westlake RJ. Functional and topological analysis of yeast acyl-CoA:diacylglycerol acyltransferase 2, an endoplasmic reticulum enzyme essential for triacylglycerol biosynthesis. J Biol Chem 2011; 286:13115-26; PMID:21321129; DOI:10.1074/jbc.M110.204412.
15. Dahlqvist A, Stahl U, Lennman M, Banas A, Lee M, Sandager L, et al. Phospholipid: diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc Natl Acad Sci USA 2000; 97:6487-92; PMID:10829075; DOI:10.1073/pnas.120067297.
16. Oelkers P, Tinkelenberg A, Erendiz N, Cromley D, Billheimer JT, Sturley SL. A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. J Biol Chem 2000; 275:15609-12; PMID:10767588; DOI:10.1074/jbc.C000144200.
17. Sandager L, Gustavsson MH, Stahl U, Dahlqvist A, Wiberg E, Banas A, et al. Storage lipid synthesis is non-essential in yeast. J Biol Chem 2002; 277:6478-82; PMID:11741946; DOI:10.1074/jbc.M109109200.
18. Jacquier N, Choudhary V, Mari M, Toulmay A, Reggiori F, Schreiter R. Lipid droplets are functionally connected to the endoplasmic reticulum in Saccharomyces cerevisiae. J Cell Sci 2011; 124:2424-37; PMID:21693588; DOI:10.1242/jcs.076836.
19. Blanchette-Mackie EJ, Dwyer NK, Barber T, Coney RA, Takeda T, Rondinone CM, et al. Perilipin is located on the surface layer of intracellular lipid droplets in adipocytes. J Lipid Res 1995; 36:1211-26; PMID:7669999.
20. Peelman F, Vinaintmont N, Verhee A, Vanloo B, Verschelde JL, Labeur C, et al. A proposed architecture for lecithin cholesterol acyl transferase (LCAT): identification of the catalytic triad and molecular modeling. Proteins Sci 1998; 7:587-99; PMID:9541390; DOI:10.1002/pro.5560070307.
21. Adimoolam S, Lee YP, Jonas A. Mutagenesis of highly conserved histidines in lecithin:cholesterol acyltransferase: identification of an essential histidine (His 377). Biochem Biophys Res Commun 1998; 243:337-41; PMID:9480810; DOI:10.1006/bbrc.1997.7995.
22. Ghosal A, Banas A, Stahl U, Dahlqvist A, Lindqvist Y, Stymne S. Saccharomyces cerevisiae phospholipid:diacylglycerol acyltransferase (PDAT) devoid of its membrane anchor region is a soluble and active enzyme retaining its substrate specificities. Biochim Biophys Acta 2007; 1771:1457-63.
23. Fisher EA, Ginsberg HN. Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. J Biol Chem 2002; 277:17377-80; PMID:12006608; DOI:10.1074/jbc.R100068200.
24. Olofsson SO, Asp L, Boren J. The assembly and secretion of apolipoprotein B-containing lipoproteins. Curr Opin Lipidol 1999; 10:341-6; PMID:10482137; DOI:10.1097/00041433-199908000-00008.
25. Hussain MM, Shi J, Dreizen P. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. J Lipid Res 2003; 44:22-32; PMID:12518019; DOI:10.1194/jlr.R200014-JLR200.
26. Khandelia H, Duelund L, Pakkanen KI, Ipsen JH. Triglyceride blisters in lipid bilayers: implications for lipid droplet biogenesis and the mobile lipid signal in cancer cell membranes. PLoS ONE 2010; 5:12811; PMID:20877649; DOI:10.1371/journal.pone.0012811.