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MINIREVIEW

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Triacylglycerol Homeostasis: Insights from Yeast*[S]

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The endemic increase in lipid-associated disorders such as obesity and type 2 diabetes mellitus has placed triacylglycerol metabolism and its associated organelle, lipid droplets, in the spotlight of biomedical research. Key enzymes of triacylglycerol metabolism are structurally and functionally conserved between yeast and mammalian cells, and studies in yeast have contributed significantly to the understanding of their biological function(s). Based on these similarities, studies performed in yeast may provide further significant mechanistic insight into the molecular basis of triacylglycerol homeostasis and its important physiological roles in healthy and diseased cells.

A central but as yet unanswered question in cell biology concerns the amount of phospholipids or TAG[2] that is required in a living cell: how is lipid homeostasis regulated in coordination with nutritional and environmental conditions? The cellular TAG content in yeast varies dramatically between different stages of growth and development (1, 2), which underscores its important metabolic role and the active involvement of its associated organelle, the LD, in cellular physiology. The key anabolic and catabolic enzymes involved in TAG metabolism are conserved between yeast and mammals (Fig. 1 and supplemental Table 1) and have recently been extensively reviewed (3–6). Therefore, this minireview will focus on the regulatory and physiological aspects of TAG metabolism in yeast and what we can potentially learn from studies in this organism about TAG homeostasis in mammalian cells.

Regulation of TAG Homeostasis

The specific requirements for storage and membrane lipids oscillate as cells grow and divide or enter the non-replicative stationary (quiescent) phase. During these periods, it appears to be crucial to control the metabolic flux of FA either into phospholipids (proliferative state) or into or out of TAG (nutrient limitation conditions). Because yeast cells typically do not feed on exogenous FA, all net cellular requirements for membrane, signaling, and storage lipids need to be satisfied by *de novo* FA synthesis, carried out by acetyl-CoA carboxylase and the FA synthase complex, encoded by the *ACC1* and the *FAS1* and *FAS2* genes, respectively. Acetyl-CoA carboxylase is the first and rate-limiting enzyme and is controlled at the transcriptional level in coordination with phospholipid synthesis (7) and by phosphorylation by Snf1p, the yeast AMP-activated protein kinase catalytic subunit and ortholog of mammalian AMPK (8). Although acetyl-CoA carboxylase activity is controlled by the Snf1p kinase, it remains to be determined whether TAG homeostasis is also directly regulated by this major energy-sensing kinase. Besides the potential impact of FA *de novo* synthesis on TAG content, its synthesis is largely controlled by the activity of the *PAH1*-encoded PA phosphatase, a heavily phosphorylated and strictly regulated protein (9–12) that is the functional ortholog of mammalian lipin (see below) (13). Yeast mutants lacking PA phosphatase are characterized by a lipodystrophy phenotype in analogy to their mouse counterparts. The regulation of DGATs, encoded by *DGA1* (functional homolog of mammalian DGAT2) and *LRO1* genes (homolog of mammalian lecithin:cholesterol acyltransferase and phosphatidylcholine:cholesterol acyltransferase and functionally characterized as a PDAT), is less clear: during logarithmic growth, PDAT appears to be more active, whereas DGAT activity may contribute more significantly to TAG formation in early stationary phase in yeast (14). The specific molecular mechanisms of regulation of these enzymes are as yet unknown.

Yeast cells entering vegetative growth display a highly stimulated turnover of TAG, which is thought to release lipid precursors for rapid initiation of (membrane) growth (1). Quantitative simulation of this initial phase of growth by dynamic flux balance analysis indeed demonstrates the requirement for TAG degradation for membrane lipid production and cell-surface expansion (2). During this adaptation to the presence of glucose, *de novo* FA synthesis appears not to be sufficient to satisfy the cellular requirements, and the TAG lipases encoded by *TGL3* and *TGL4* genes are most active, leading to almost complete TAG degradation (see below) (1, 6). The *TGL4*-encoded lipase is the functional ortholog of mammalian adipose triglyceride lipase (desnutrin, PNPLA2 (patatin-like phospholipase domain-containing protein 2)) (1). Because formation and function of peroxisomes, the only site of β-oxidation in yeast, are repressed by glucose, it is evident that lipolysis serves another purpose in addition to releasing FA for energy production during that phase of growth. Both quantitative modeling and microscopic evidence suggest that replenishment of TAG pools already takes place while lipolysis is still active in cells entering vegetative growth.

At the cellular level, TAG synthesis and degradation indeed oscillate in coordination with the cell division cycle: Tgl4p lipase-dependent TAG breakdown is activated by

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[2] The abbreviations used are: TAG, triacylglycerol(s); LD, lipid droplet(s); FA, fatty acid(s); AMPK, AMP-activated protein kinase; PA, phosphatidic acid; DGAT, acyl-CoA:diacylglycerol acyltransferase; PDAT, phospholipid:diacylglycerol acyltransferase; ACSL, long chain acyl-CoA synthetase; ER, endoplasmic reticulum; DAG, diacylglycerol; PC, phosphatidylcholine.

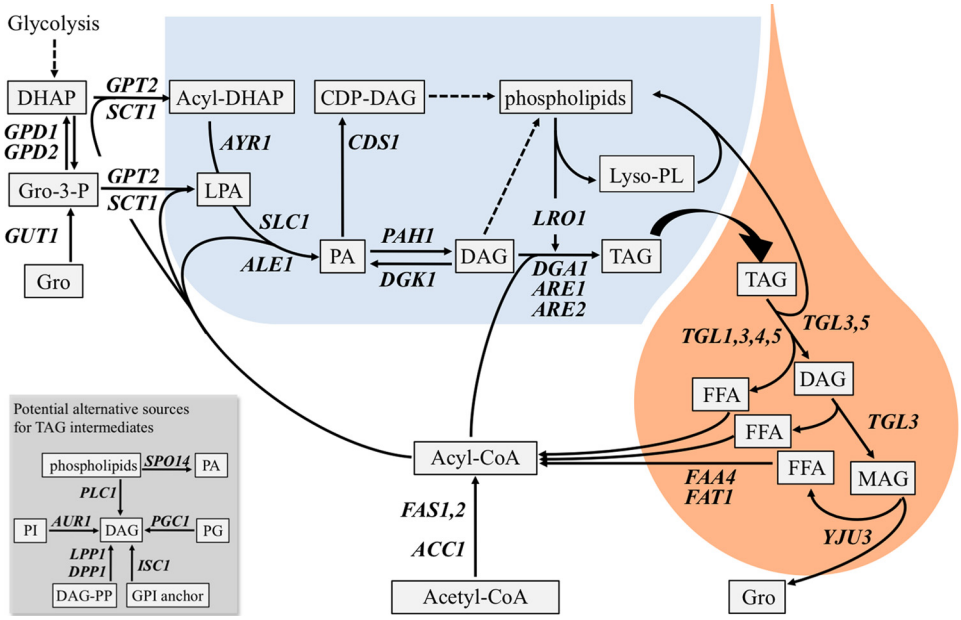


FIGURE 1. **Enzymes involved in TAG homeostasis and their spatial organization.** Gene names (*italicized*) and their functions are provided in supplemental Table 1 and in text. The area in *light blue* indicates the ER; the area in *red* indicates LD. The *inset* in *gray* indicates alternative pathways for the synthesis of potential TAG precursors; their specific contribution to TAG formation is unclear. *Dashed lines* indicate multiple enzymatic steps. *FFA*, free FA; *LPA*, lysophosphatidic acid; *DAG-PP*, DAG pyrophosphate; *MAG*, monoacylglycerol; *Gro*, glycerol; *DHAP*, dihydroxyacetone phosphate; *PL*, phospholipid; *PI*, phosphatidylinositol; *PG*, phosphatidylglycerol; *GPI*, glycosylphosphatidylinositol. Note the dual functionality of enzymes encoded by *TGL3* and *TGL5* genes as TAG lipases and TAG:phospholipid acyltransferases (18).

CDK1 (cyclin-dependent protein kinase 1)/Cdc28p at the G_1/S transition, which is characterized by the morphogenesis checkpoint and bud emergence. Lipolysis contributes to bud formation, presumably by providing precursors for membrane or signaling lipids (15); in the absence of lipolysis, cell cycle progression is significantly delayed and becomes solely dependent on *de novo* FA synthesis. Conversely, TAG synthesis is down-regulated by CDK1/Cdc28p-dependent phosphorylation of PA phosphatase (16, 17). Notably, phosphorylation/inhibition of this enzyme occurs at G_2/M of the cell cycle, suggesting that TAG formation and lipolysis operate in parallel from G_1/S until G_2/M, albeit at different subcellular locations. The reason for this apparent discrepancy may reside in the essential cellular requirement to tightly control the metabolic flux of FA into TAG or phospholipids during cellular growth. In that respect, it deserves mentioning that TAG lipases encoded by *TGL3* and *TGL5* genes may also act as transacylases, independent of their "pure" lipolytic activity, supporting their important function also to control phospholipid acyl composition (18).

Complete TAG breakdown generates glycerol and free FA that may be reactivated to acyl-CoA by ACSLs, of which six activities are known in yeast (19) and which can be functionally complemented, at least in part, by the mammalian orthologs (19, 20). In contrast to mammalian cells, FA synthase in yeast releases acyl-CoA rather than free FA, which makes the yeast ACSL activities dispensable for utilizing *de novo* synthesized FA. Notably, the two *FAA4*- and *FAT1*-encoded ACSL enzymes are localized to LD, *i.e.* in close proximity to the site of TAG storage and lipolytic action (21, 22). It is tempting to speculate that these ACSL activities are therefore metabolically coupled to lipolysis, *i.e.* for channeling free FA released from TAG (or steryl ester) breakdown toward activation and further metabolic utilization.

Spatial Organization of TAG Metabolism

The apparent futile cycle of anabolic and catabolic processes of TAG homeostasis acting in parallel during the cell division cycle (15, 17) is perhaps better understood in view of the spatial separation of the biosynthetic and lipolytic processes. Most of the TAG biosynthetic steps occur in the ER, including the *SCT1*-, *GPT2*-, *SLC1*-, and *ALE1*-encoded acyltransferases (Fig. 1). However, more subtle localization patterns of the glycerol-3-phosphate acyltransferases Sct1p and Gpt2p to distinct ER subfractions may also exist (23).

PA phosphatase, which generates DAG, is associated with membranes (presumably the ER) in a salt-extractable form and may also be present in the cytosol and in the nucleus (11), where it may act as a repressor of phospholipid biosynthetic genes. PDAT, encoded by *LRO1*, localizes to the ER, consistent with its preferred utilization of PC as the acyl donor (24). On the other hand, DGAT, encoded by *DGA1*, localizes to both the ER and LD in yeast (21, 22, 25). Thus, it appears that this enzyme moves along with the nascent LD that is believed to emerge from the ER membrane (26, 27). The dual localization to the ER and LD of ergosterol biosynthetic enzymes encoded by *ERG1*, *ERG6*, and *ERG7*, the FA-activating enzymes encoded by *FAA4* and *FAT1*, and the lyso-PA acyltransferase encoded by *SLC1* further underscores the close functional relationship between the ER and LD, in particular with respect to lipid fluxes (5, 6, 28–30). The specific mechanisms that direct and sequester these proteins to the (nascent) LD are unknown but may involve hydrophobic interactions with multiple protein domains rather than specific LD "targeting" sequences (31).

Notably, all the enzymes identified so far localizing to both the ER and LD catalyze anabolic enzymatic steps, whereas the catabolic enzymes, such as the TAG lipases and steryl ester hydrolases encoded by *TGL3*, *TGL4*, *TGL5*, and *TGL1* and by *YEH1*, respectively, are restricted to the LD (1, 5, 6, 30). This differential localization for anabolic and catabolic enzymes suggests different routes for their LD association either through sequestration from the cytosol or via targeting to the ER. It should also be noted that yeast (or bacterial) LD do not appear to contain homologs of mammalian perilipins ("PAT proteins" (32): perilipin, ADRP (adipose differentiation-related protein), TIP47 (tail-interacting protein of 47 kDa), S3–12, and OXPAT), which regulate cellular lipid stores, *e.g.* by mediating phosphorylation-dependent access of lipases to the LD surface.

Yeast TAG lipases encoded by *TGL3*, *TGL4*, and *TGL5* are constitutively present on LD and do not alter their localization during periods of lipolysis or lipogenesis (15). Thus, the lipolytic process in yeast is largely regulated by direct activation or inactivation of these proteins on LD.

Physiological Importance of TAG

TAG were in the past considered mainly as an efficient storage form of FA that may serve as energy substrates in the absence of other carbon sources. This view has considerably changed during the last few years, with emerging evidence that TAG serve specific metabolic functions. In mammalian cells, TAG formation was found to be crucial for the detoxification of lipotoxic FA (33, 34). Similarly, yeast mutants that are lacking all four acyltransferases involved in TAG formation are highly sensitive to unsaturated FA supplementation (35, 36). In the absence of TAG synthesis, oleic acid supplementation leads to a rapid block of the secretory pathway at the level of the ER, up-regulation of the unfolded protein response, and ultimately cell death (36). Multiple genes involved in iron and phospholipid metabolism are repressed in the absence of TAG synthesis (35). Notably, saturated FA are not toxic to wild-type yeast or to TAG-deficient mutants and may indeed suppress unsaturated FA-induced lipotoxicity in such mutant strains by generating a more balanced FA composition in cellular phospholipids (36). Unsaturated FA-induced lipotoxicity in yeast mutants lacking TAG is also suppressed by expression of the human diacylglycerol acyltransferase DGAT2, providing an intriguing model system for functional studies of the heterologous enzyme in yeast (35).

In addition to providing a buffer for excess FA detoxification, TAG also provide important metabolites for sporulation or cell cycle progression (15, 18). The specific nature of the lipid-dependent cell cycle checkpoint is currently unclear, but the temporal coincidence of lipolysis requirements with bud emergence in the cell division cycle indicates the involvement of the major morphogenesis checkpoint regulator, the Swe1p protein kinase, in this process. The TAG-derived lipid species required to "grease" the cell cycle are unknown (15). Multiple homozygous diploid lipase-deficient mutants are unable to sporulate, demonstrating the importance of TAG degradation for providing energy substrate for peroxisomal β-oxidation or for phospholipid remodeling in the context of spore membrane formation (18).

Coordination of TAG Homeostasis with Other Cellular Pathways

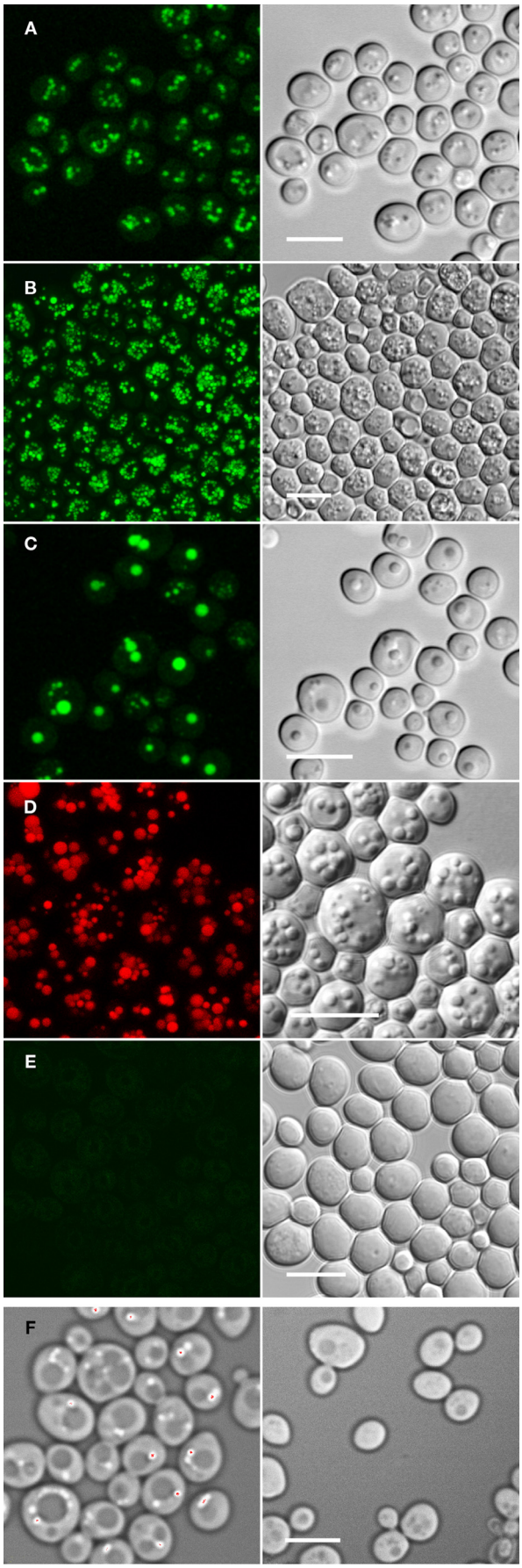
Because TAG and phospholipids share common precursors, it is evident that shifting the balanced synthesis either way will affect the steady-state concentration of the other components. For instance, defective PC synthesis in mutants lacking the *CHO2*- or *OPI3*-encoded phosphatidylethanolamine and phospholipid methyltransferases, respectively, results in increased cellular TAG content (37). The potential cross-talk between the Kennedy (CDP-choline) pathway and TAG metabolism is less clear: in the presence of choline or ethanolamine, DAG resulting from lipolysis may potentially be directly utilized for phospholipid synthesis, which would involve a DAG translocation step from the LD to the ER for its incorporation into phospholipid. However, the position and stereoselectivity of the yeast lipases have not been determined yet, and it is unclear at present if indeed the phospholipid precursor *sn*-1,2-DAG or rather *sn*-1,3- or *sn*-2,3-DAG species or a mixture thereof is generated by TAG lipolysis. Alternatively, utilization of DAG for phospholipid synthesis in the presence of ethanolamine or choline may compete with TAG formation; however, no evidence for either aspect is currently available.

Even less evident is the connection between TAG accumulation in mutants lacking the yeast tafazzin ortholog encoded by the *TAZ1* gene, which harbors monolysocardiolipin acyltransferase activity required for mitochondrial cardiolipin remodeling (38, 39). Mutations in this gene in humans result in a severe disease termed Barth syndrome. It was speculated that lack of this activity may increase PC and DAG levels, giving rise to increased TAG synthesis (38).

In addition to direct metabolic connections, such as competing transacylation processes, other "physiological" or regulatory processes are likely to control TAG homeostasis. For instance, a block of the secretory pathway that is induced by inactivating the COPII component Sec13p involved in ER-to-Golgi trafficking leads to dysregulation of phospholipid synthesis and concomitant TAG accumulation; thus, TAG synthesis may function as an alternative "exit" for excess lipid intermediates that are not disposed off from the ER through the secretory pathway (40). Other membrane trafficking pathways may affect TAG homeostasis in a similar manner.

Identification of Novel Regulators of TAG Homeostasis

The storage compartment for TAG, the LD, interacts with numerous other cellular organelles and processes (41), and multiple experimental approaches are undertaken to identify and characterize novel regulators of LD/TAG homeostasis. Microscopic analyses are particularly powerful to identify mutants with altered LD and TAG content. Fig. 2 illustrates the LD phenotypes of wild-type yeast and various defective mutants: *snf1* mutants lack the yeast AMPK and harbor hyperactive acetyl-CoA carboxylase (8), leading to FA overproduction and subsequent TAG accumulation; *fld1* (few lipid droplets) mutants lack the yeast seipin ortholog (42, 43) and are characterized by abnormally shaped LD; *tgl3 tgl4* mutants lack the major TAG lipases encoded by the genes *TGL3* and *TGL4*, which results in "obese" cells (1); and a quadruple mutant lacking *DGA1*, *LRO1*, *ARE1*, and *ARE2* genes encoding DAG acyltransferase activities is devoid of TAG and steryl esters and of LD altogether (35, 36). Even subtle changes in cellular TAG content or LD morphology may become easily microscopically visible or result in changes of biophysical properties of the cells. In an attempt to isolate mutants with increased TAG content based on flotation, Kamisaka *et al.* (44) identified, among other factors, the protein Snf2p, which is the catalytic subunit of the SWI/SNF chromatin-remodeling complex, as a potential regulator of lipid metabolism. A physiological link between lipid homeostasis and chromatin structure is intriguing and deserves further attention. Imaging-based screens of the entire yeast deletion collection comprising some ~4700 viable mutants and making use of the lipophilic dyes Nile red (42) and BODIPY 493/503 (43) yielded a large number of factors regulating LD



content and morphology. The most prominent hit in both screens identified mutants that lacked the gene *FLD1*, which is a functional ortholog of human *BSCL2*, implicated in a severe inherited disease termed Berardinelli-Seip congenital lipodystrophy type 2 (Fig. 2*C*). Indeed, heterologous expression of the wild-type *BSCL2* gene, but not of mutant variants, restored wild-type LD morphology to a yeast *fld1* mutant, demonstrating the functional conservation of the protein (42). Further factors with a potential role in neutral lipid metabolism resulting from these imaging-based screens include endosomal/vacuolar and mitochondrial proteins but also kinases and transcription factors (41–43). It should be noted that there was surprisingly little overlap (~10%) among the mutants identified in both mutant screens, which suggests that (*a*) these screens are far from being saturated and (*b*) LD morphology is very dynamic and strongly dependent on the respective growth and analysis conditions. Thus, the chapter of identifying all the relevant components involved in TAG metabolism and LD biogenesis is far from being complete and may hold many more surprises.

Outlook

The commonly used term "neutral lipid" to describe the major LD components steryl esters and TAG solely reflects their biophysical property as being uncharged but by no means does justice to their biological functions. TAG metabolism instead actively participates in vital cellular processes. The conservation of key metabolic steps makes yeast an intriguing model for functional analyses of heterologously expressed mammalian genes involved in TAG metabolism. Studies in yeast also contribute to understanding complex metabolic networks, such as those involved in nutrient and lipid signaling, lipotoxicity, and the metabolic syndrome (45, 46). Notably, some key enzymatic functions were first identified and characterized in yeast at the molecular level, such as the PA phosphatase activity of lipin (12), a mammalian lipodystrophy factor for many years in search of a biochemical function (13). Replacement of the yeast orthologs by their mammalian counterparts, either wild-type or mutant forms derived from patients, has proven to be a viable strategy to assess biological activity in an *in vivo* setting that provides an impressive arsenal of genetic, genomic, and cell biological tools for functional studies (35, 42).

FIGURE 2. **Analyzing LD morphology as an indicator of TAG homeostasis using different staining and microscopic techniques.** *A–E*, *left panels*, fluorescence; *right panels*, transmission (differential interference contrast). *Scale bars* = 10 μm. *A*, LD visualization in wild-type cells using a green fluorescent protein-tagged reporter construct (1). *B*, accumulation of LD in an *snf1* deletion strain that displays hyperactive Acc1p (8) and TAG accumulation. Note that the size of LD does not increase in this mutant but rather their number (BODIPY 493/503 staining). *C*, morphologically altered LD in an *fld1* mutant (42, 43) lacking the yeast ortholog of mammalian BSCL2, implicated in Berardinelli-Seip congenital lipodystrophy type 2 (BODIPY 493/503 staining). *D*, LD accumulation in an obese yeast mutant lacking the two major TAG lipases, Tgl3p and Tgl4p (Nile red staining) (1). *E*, a quadruple mutant lacking the four acyltransferases involved in TAG synthesis (35, 36) that also lacks LD and any detectable Nile red staining in the 550–570-nm emission range (see Ref. 36 for experimental details). *F*, coherent anti-Stokes Raman scattering microscopy of a wild-type strain (*left panel*) and the quadruple mutant (*right panel*). Coherent anti-Stokes Raman scattering enables label-free detection of LD based on the spectroscopic properties of lipid molecules. *A–C* and *E* are courtesy of Heimo Wolinski (University of Graz); *F* is courtesy of Lu Fake and Huang Zhiwei (National University of Singapore).

Now that the basic enzymatic steps involved in TAG homeostasis have been worked out, what are the next challenges? Why does TAG homeostasis require so many redundant activities, such as acyltransferases and lipases? Obviously, these enzymes display distinct substrate specificities that may be relevant in the context of maintaining cellular FA and phospholipid homeostasis and regulation under different environmental, nutritional, and developmental conditions. The most intriguing problems concern the biogenesis of the organelle that accommodates TAG and steryl esters, the LD, and the functional interplay between the ER and LD with respect to controlling lipid fluxes. These "great balls of fat" were only recently recognized as a highly dynamic cellular compartment (41, 47–49), which is now subject to extensive studies in microorganisms, invertebrates, and mammalian and plant cells. Imaging-based large-scale functional genomic screens performed in various cell types, including yeast, are likely to uncover the critical factors required for LD formation, morphology, catabolism, and inheritance (27, 42, 50). The combination of such refined imaging-based screens in different cell types with proteomic and lipidomic analyses of isolated LD is expected to uncover and converge at a critical and conserved set of proteins and lipids relevant for LD biogenesis and metabolism in eukaryotes. These studies will also contribute to solving the puzzles as to the highly redundant activities involved in TAG formation and breakdown and their relevance for cellular physiology and disease.

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