thematic review

*Thematic Review Series: Proteomics*

# Demonstrated and inferred metabolism associated with cytosolic lipid droplets

**Joel M. Goodman**[1]

Department of Pharmacology, University of Texas Southwestern Medical School, Dallas, TX 75390-9041

**Abstract** **Cytosolic lipid droplets were considered until recently to be rather inert particles of stored neutral lipid. Largely through proteomics is it now known that droplets are dynamic organelles and that they participate in several important metabolic reactions as well as trafficking and interorganellar communication. In this review, the role of droplets in metabolism in the yeast *Saccharomyces cerevisiae*, the fly *Drosophila melanogaster*, and several mammalian sources are discussed, particularly focusing on those reactions shared by these organisms. From proteomics and older work, it is clear that droplets are important for fatty acid and sterol biosynthesis, fatty acid activation, and lipolysis. However, many droplet-associated enzymes are predicted to span a membrane two or more times, which suggests either that droplet structure is more complex than the current model posits, or that there are tightly bound membranes, particularly derived from the endoplasmic reticulum, which account for the association of several of these proteins.—** Goodman, J. M. **Demonstrated and inferred metabolism associated with cytosolic lipid droplets. *J. Lipid Res.* 2009.** 50: **2148–2156**.

Cytosolic lipid droplets, originally thought to be simply coalesced neutral lipids waiting for lipolysis at metabolic demand, are now known to be considerably more complicated both structurally and functionally. There is general agreement that droplets are comprised of a core of neutral lipids, principally triglycerides and steryl esters, surrounded by a leaflet of phospholipids into which are embedded a specific subset of cellular proteins, the most abundant of which are members of the PAT family (see below) in animal cells (1). However, this model is probably too simple; there is evidence from physical probes of droplets isolated from yeast mutants unable to synthesize triglycerides or steryl esters that these two molecular families are partially segregated within the core, with thin shells of steryl esters forming concentric hollow spheres around an inner core composed principally of triglycerides (2).

The next layer of complexity is the functional inhomogeneity of droplets. Subsets of droplets within the same cells exist with different populations of PAT proteins, differentiating among different sizes, ages, and levels of metabolic activity (3, 4). Perhaps most surprisingly, droplets may be comprised, at least in some cases, not of the layered core-phospholipid shell architecture at all but a knot of tightly woven endoplasmic reticulum (ER) surrounded by secreted neutral lipid, itself encased with a single leaflet. Such a model is based on electron microscopic thin sections (5), freeze fracture-immunogold evidence (6), immunohistochemical studies of ER luminal proteins within the droplet (7), and the identification of these proteins, notably ER chaperones, in several proteomic studies. Although certainly, such a complex structure must obey physical laws governing aqueous interactions with hydrophobic lipids and artifacts in processing for electron microscopy do occur, it may be best at present to keep an open mind and consider that droplets may not have the same structure among tissues and that they may take multiple physical forms in rapid order as they dynamically perform their functions.

What are these functions? The most obvious one is lipid metabolism, namely the biogenesis and breakdown of the neutral lipids contained within the droplet. Although this conclusion predates proteomic studies (8), these recent studies have revealed the breadth and conservation of metabolic reactions that occur at or near the droplet surface, the subject of this review. Moreover, proteomics has demonstrated the surprising fact that droplets are likely to be very active in organellar communication because they are replete in rab proteins and other trafficking molecules. Our knowledge from proteomic studies of droplet

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Abbreviations: ATGL, adipocyte triglyceride lipase; CHO, Chinese hamster ovary; DAG, diacylglycerol; ER, endoplasmic reticulum; GFP, green fluorescent protein; 17HSD, 17β-hydroxysteroid dehydrogenase; HSL, hormone-sensitive lipase; MAM, mitochondrially associated membrane; PKA, protein kinase A; TAG, triacylglycerol; TGL, triglyceride lipase.

[1]To whom correspondence should be addressed.
e-mail: Joel.Goodman@UTSouthwestern.edu

trafficking and communication is discussed separately in this thematic review series.

A major caveat must be kept in mind when evaluating droplet proteomics data: besides droplet trafficking through transient interactions with vesicles or target organelles such as early endosomes (9), droplets make extensive, tight, and long-lasting synapses with the endoplasmic reticulum, mitochondria, and peroxisomes (10, 11). The fact that ER, mitochondrial, peroxisomal, and a few plasma membrane proteins are found with such high frequency in the droplet proteome probably reflects these tight interorganellar interactions, perhaps similar to the mitochondrially associated membranes (MAMs) that link mitochondria with ER (12). The molecular basis for droplet-mediated synapses are not yet known. Besides the frequent occurrence of specific nondroplet organelle proteins in the droplet proteome, adventitious contamination of droplets is unlikely in view of the unique density of droplets that allow their flotation to the top of aqueous buffers and density gradients after centrifugation while all other cell components sink (which also permits several washes with high recovery), and the nonrandom coisolation of subsets of proteins from other organelles, such as the β-oxidation peroxisomal enzymes (10), which suggests specialized regions for metabolically-productive droplet interactions at the synapses.

Droplet-ER interactions are a special case; it is the rule rather than the exception that enzymes of lipid metabolism that are found in the droplet proteome are also found to varying extents in the ER. This has been well documented in yeast through genome-wide green fluorescent protein (GFP)-tagging (13, 14). Erg6p, an enzyme in the latter part of the ergosterol biosynthetic pathway, is the only droplet protein in the pathway with a near-exclusive droplet localization in yeast; Erg1p, Erg7p, and Erg 27p are dually localized, and the pattern changes depending on metabolic state. Whether this general rule is specific for yeast, in which droplets remain on the ER surface (15), is not yet clear. However, several examples already exist in mammalian cells: cytochrome b5 reductase (DT diaphorase) and various sterol dehydrogenases (see **Table 1**), were classically considered ER proteins. Many enzymes of sterol metabolism that appear in droplet proteomes have multiple membrane spans and it is difficult to imagine them arranged in the single leaflet surrounding a hydrophobic core of neutral lipids. A solution to this problem, besides that of invoking internal ER cisternae within droplets, is to consider these enzymes in a specialized ER compartment that is very close to, and tightly bound with, the droplet (a "droplet synapse") that separates from the bulk ER during fractionation, copurifying with droplets. If this structure resembles that of MAMs in contact with mitochondria, it would explain the frequent coisolation of ER luminal chaperones with droplets because chaperones such as luminal HSP70 are directly involved in MAM structure (12).

The metabolic functions of droplets, as revealed or confirmed by proteomic studies, can be grouped into fatty acid synthesis and activation, sterol biosynthesis, triglyceride biosynthesis, and fatty acid mobilization from sterol esters and triglycerides. Table 1 lists the identified droplet enzymes in these pathways as found by proteomics technology in *Saccharomyces cerevisiae*, *Drosophila melanogaster*, rodents, and humans. The ones that are repeatedly found with droplets are discussed below.

## FATTY ACID SYNTHESIS AND ACTIVATION

The first step in fatty acid synthesis is the generation of malonyl-CoA, catalyzed by acetyl-CoA carboxylase, the rate-limiting enzyme in the pathway. The multi-domain fatty acid synthase (FAS) generates palmitic acid (slightly longer chains in yeast) from acetyl-CoA and malonyl-CoA. Most of the carboxylase and FAS are cytosolic although FAS is detected in the droplet proteome in flies (Table 1). Further elongation of palmitic acid and fatty acid desaturation occurs by enzymes in the ER in mammals and probably in yeast (16). Finally, fatty acids must be activated to CoA thioesters to be oxidized in the mitochondria and peroxisomes or added to glycerol or sphinganine to form triglycerides or sphingolipids, respectively. Both acetyl-CoA carboxylase and acyl-CoA synthetases have been identified in several droplet proteomes (Table 1).

Although much of acetyl-CoA carboxylase localizes in the cytosol, there is some controversy about the localization of the particulate form. Early reports suggested that a fraction of mammalian acetyl-CoA carboxylase was associated with mitochondria or peroxisomes [references contained within (17)] but later work did not confirm these observations in rat liver; on the contrary, evidence involving cytoskeletal agents suggested that some of the enzyme was associated with microtubules (17). In *S. cerevisiae*, the protein was originally localized to the surface of the ER (18). Later work from the same group indicated that in well-oxygenated growing cultures the protein is cytosolic, whereas in nutrient-limited conditions it appears close to mitochondria (16) and has been found in the yeast mitochondrial proteome (19). Caution must be brought to evaluating localization of tagged carboxylase because fusions at the carboxy terminus may not rescue the null mutant (16). The appearance of acetyl-CoA carboxylase in the droplet proteome may be the result of association of other organelles with droplets, although because the enzyme is classically negatively regulated by fatty acids, it is tempting to speculate that this association occurs on the droplet surface. A study of the effects of incubation with oleic acid (making fatty acid synthesis redundant) on droplet localization of acetyl-CoA carboxylase has not been performed.

Elongation of fatty acids beyond palmitate in mammals and perhaps in yeast (16) is promoted by elongase and desaturase enzymes localized in the ER, requiring NADPH. This source of reducing equivalents can be provided by cytochrome b5 reductase (Cyb5r) (20). Although elongases are usually not found in the droplet proteome, Cyb5r3 (diaphorase 1) is often found in mammalian droplet proteomes. This enzyme also is important in shuttling reducing equivalents in microsomal fatty acid desaturation

TABLE 1. Metabolic functions of droplets as revealed by proteomics

| Protein | Reference(s) | Comments |
|---|---|---|
| | **Fatty Acid Synthesis** | |
| ATP citrate lyase | (e) | Generates acetyl-CoA |
| Acetyl-CoA carboxylase/ACC1 | (i) (j) (n) (o) (e) | Generates malonyl CoA |
| 3-Oxoacyl(ACP) synthase | (e) | *Drosophila*; early step in FA synthesis |
| Fatty acid synthase | (e) | *Drosophila* |
| Diaphorase 1/Cytochrome b5 reductase | (g) (h) (j) (l) (n) (o) | Redox carrier in FA elongation and many others |
| Fatty acid desaturase 2 | (e) (m) | Many hydrophobic spans likely |
| | **Fatty Acid Activation** | |
| Acyl-CoA synthetase/ACSL1 | (g) (n) | Fatty acid-CoA ligase |
| Acyl-CoA synthetase/ACSL3 | (g) (h) (i) (j) (l) (n) (o) | Fatty acid-CoA ligase |
| Acyl-CoA synthetase/ACSL4 | (g) (h) (j) (l) (n) | Fatty acid-CoA ligase |
| Acyl-CoA synthetase/ACSL5 | (m) | LACS2 |
| Acyl-CoA synthetases/FAA1, FAA4, FAT1 | (a) (d) | Yeast enzymes; FAT1 is a FA transporter; may have synthetase activity |
| | **Steroid Synthesis** | |
| Squalene epoxidase/ERG1 | (a) (i) (j) (o) (d) | |
| Lanosterol synthase/ERG7 | (a) (g) (h) (i) (j) (m) (o) (d) | |
| NAD(P) steroid dehydrogenase like (NSDHL)/ERG26 | (g) (h) (i) (m) (o) | Sterol synthesis |
| 3-keto reductase 17βHSD7/ERG27 | (b)*(c)*(g) (j) (n) (o) (d) | Sterol synthesis |
| C24-methyltransferase/ERG6 | (a) (c)* (d) | Specific to ergosterol synthesis in fungi |
| 17β-HSD11 (retinal short chain dehydrogenase) | (h) (i) (j) (l) (m) (n) (o) (e) | Testosterone biosynthesis; steroid metabolism |
| 17β-HSD4 | (l) | Bile salt snthesis |
| 17β-HSD13 | (m) | A short-chain dehydrogenase |
| 17β-HSD3 | (m) | Steroid metabolism |
| | **Triglyceride Synthesis** | |
| AcylDHAP reductase/AYR1 | (d) | Determined early biochemically (68) |
| LysoPA acyltransferase/SLC1 | (d) | Determined earlier biochemically (69) |
| DAG acyltransferase/DGA1 | | Determined biochemically in yeast (70) |
| | **Lipolysis** | |
| Hormone-sensitive lipase | (f) (g) | Diglyceride lipase [first characterized in (71)] |
| Fat-specific gene 27 | (g) | Lipase activity |
| ATGL | (n) (o) | Triglyceride lipase |
| Monoglyceride lipase | (m) | |
| Tgl3, Tgl4, Tgl5 | (a) | Yeast triglyceride lipases [for Tgl4 and 5 see (60)] |
| Tgl1p, Yeh1p | (a) | Yeast steryl ester lipases; Yeh1 localized in (62) |
| PLC α | (n) | |
| Phospholipase A1 | (n) | |
| | **Lipase Modulators** | |
| Perilipin | (g) | PAT family |
| ADRP | (g) (h) (i) (k) (l) (m) (n) (o) | PAT family |
| TIP47 | (g) (h) (l) (m) (o) | PAT family |
| S3-12 | (g) | PAT family |
| LSD2 | (e) (f) | PAT family (*Drosophila*) |
| CGI-58 | (g) (i) (n) (o) (f) | Regulator of ATGL; has endogenous acyltransferase activity (72) |
| Caveolin 1 | (g) (m) (n) | May bridge perilipin with PKA to stimulate lipolysis |
| | **Other Redox Enzymes** | |
| Cytochrome p450 | (e) | Mostly in ER |
| Cytochrome b5 | (e) | Mostly in ER |
| Alcohol dehydrogenase 4 | (j) (m) (n) (e) | Most in cytoplasm. Broad specificity, including retinols, aliphatic alcohols, and steroids |
| Aldehyde dehydrogenase /ALDH3B1 | (g) | Can oxidize medium and long chain aldehydes |
| Glyceraldehyde phosphate dehydrogenase | (a) (h) (l) (m) (n) (o) (e) | Cytosolic glycolytic enzyme, but often found with droplets |
| Xanthine oxidoreductase | (k) | Identified in mammary tissue only |
| Gulonolactone oxidase | (m) | *Drosophila*; missing in humans. Role in ascorbic acid synthesis |
| Short-chain dehydrogenase/reductase member 1 | (g) (j) (n) (e) | Unknown substrate |

TABLE 1. *Continued.*

| Protein | Reference(s) | Comments |
|---|---|---|
| | **Other Enzymes** | |
| Acyl-CoA:ethanol o-acyltransferase /EHT1 | (a) (d) | Generation of medium-chain ethyl esters |
| SCCPDH (CGI49) | (h) (n) (o) | Degradation of lysine |
| PI4 phosphatase/SAC1 | (n) | |
| Serine palmitoyltransferase subunit 1 isoform a | (n) | Sphingolipid synthesis |
| SAM-dependent methyltransferase | (j) | Biosynthesis of phosphatidylcholine |
| | **Possible Contamination** | |
| Sterol carrier protein 2-related form | (l) (e) | May have thiolase activity. Peroxisomal contamination? |
| Palmitoyl-protein thioesterase | (j) (n) | Lysosomal contamination? |
| ER carboxyesterase | (k) | Mammary; used to make triglyc for lipooproteins |
| ATPsynthase2 | (g) | Mitochondrial contamination |
| Carbamoyl P Synthetase 1 | (m) | Mitochondrial contamination |
| Pyruvate carboxylase | (g) (k) (e) | Mitochondrial contamination? |
| Fatty acid translocase/CD36 | (g) | Plasma membrane contamination? |
| Lipoprotein lipase (LPL) | (g) | Plasma membrane contamination |

*Non proteomics screens.

(a) (29).

*(b) (GFP screen) (13).
*(c) (GFP screen) (14).
(d) (10).
(e) (73).
(f) (74).
(g) (23).
(h) (75).
(i) (76).
(j) (24).
(k) (77).
(l) (78).
(m) (79).
(n) (40).
(o) (5).

and P450-catalyzed reactions, including sterol synthesis and drug metabolism. An N-terminal hydrophobic sequence in the enzyme (amino acids 1–28) is sufficient for targeting the protein to droplets (21).

Reactions involving redox mechanisms are performed by several enzymes in the droplet proteome, especially those involved in sterol biosynthesis and interconversions. It is interesting to consider the possibility that glyceraldehyde-phosphate dehydrogenase, found in the droplet proteomes of yeast, flies, and mammals and considered to be a cytosolic contamination, may participate by donating electrons in some of these reactions of fatty acid synthesis and other pathways.

Activation of fatty acids with CoA is a necessary step both for oxidation and fatty acylation of glycerol and sterols. There are five subfamilies of acyl-CoA synthetases in mammals, depending mainly on fatty acid chain length of their substrates. Two members of the long chain family, ACSL3 and ACSL4, have been identified repeatedly in the mammalian droplet proteome, whereas ACSL1 and ACSL5 have been reported rarely. Among the 5 ACSL isozymes, ACSL1 is most abundant in adipose tissue. It has been localized to plasma membrane, cytosol, ER, and mitochondria (22), besides the droplet. It has been identified on lipid droplets from basal and induced 3T3-L1 cells (23) and in Chinese hamster ovary (CHO) K2 cells (24). In plasma membrane, there is evidence that ASCL1 function is coupled with the FATP importer (25). However, there was no change in fatty acid import in an ACSL1 knockdown in induced 3T3 L1 cells (26). Instead, the reacylation of fatty acids liberated from droplets was severely inhibited, providing functional evidence for a role of ACSL1 on or near droplets.

ACSL3 and ACSL4 were also detected in droplets from CHO K2 cells (ACSL3 listed as fatty acid CoA ligase) (24) and from 3T3 L1 adipocytes but not droplets from unstimulated cells (26). ACSL3 has also been localized to lipid rafts and ASCL4 to MAMs and peroxisomes from rats treated with gemfibrozil, a peroxisomal proliferating agent (27). However, the relative amounts of ACSL3 or ACSL4 that associate with droplets compared with other organelles or the physiological significance of these associations are not known. The two enzymes have different substrate preferences; ACSL3 prefers myristate, arachidonate, and eicosapentaenoate whereas ACSL4 prefers arachidonate (28).

The yeast droplet proteome contains the acyl-CoA synthetases FAA1 and FAA4 (29). FAA1 also localizes to mitochondria based on proteomics (19). A FAA4-GFP fusion protein, driven by the endogenous promoter, localizes

mainly to droplets (and also to ER) in log phase but exclusively to droplets in stationary phase (www.yeastgenome.org). FAT1, which is involved in fatty acid transport across the plasma membrane and may contain intrinsic very-long-chain acylCoA ligase activity as well (30), is also in the yeast droplet proteome (29). A strongly expressed FAT1-GFP construct localizes to droplets and ER rather than plasma membrane (14). Not surprisingly, considering its known function in the plasma membrane, hydrophobicity analysis and topology mapping demonstrate that FAT1 is an integral membrane protein (31), which would be incompatible with the droplet monolayer. Its appearance in droplets may indicate droplet binding to plasma membrane fragments containing FAT1. Perhaps there is trafficking to droplets of a truncated FAT1 protein missing the transmembrane domains.

In summary, there is good evidence that both the rate-limiting enzyme in fatty acid biosynthesis and fatty acid activation enzymes partially localize to droplets. Yeast FAA4 is unusual among these enzymes in that droplet association depends on growth conditions.

## STEROL BIOSYNTHESIS

Proteomics studies have identified several enzymes of postsqualene sterol biosynthesis that associate with droplets, suggesting that the droplet has a significant role in both the biosynthesis of cholesterol and steroid metabolism.

Two enzymes that sequentially convert squalene to lanosterol are colocalized in ER and droplets. Squalene epoxidase (yeast ERG1), which converts squalene to 2,3-oxidosqualene, was the first enzyme in the sterol biosynthetic pathway that was detected in mammalian and yeast droplets by proteomics. It is traditionally a microsomal enzyme (32). A possible reason for colocalization was provided by yeast studies, where a GFP-ERG1 fusion protein was found both in the ER and lipid droplets (14). Interestingly, only the ER-localized enzyme was active (33); however, when droplets containing inactive ERG1 are combined with ER from an ERG1 knockout strain, the activity of the epoxidase on the droplet was partially restored, suggesting that the droplets sequester the protein in an inactive form (33). Squalene epoxidase, a flavoprotein, requires a reductase such as NADPH cytochrome P450 reductase for redox cycling (34). Perhaps sequestration in droplets separates the epoxidase from the reductase, thereby regulating the enzyme. The mammalian epoxidase also requires a cytosolic squalene transfer protein (35). Access to this factor may be lost on the droplet.

Lanosterol synthase (ERG7) has also been localized to droplets, both in the basal and stimulated state in 3T3-L1 adipocytes (23), as well as in yeast. In yeast, ERG7-GFP is predominantly on droplets with less staining in the ER (13, 14). Unlike ERG1, most enzyme activity of the native protein in yeast is associated with droplets, not ER (36).

During the latter steps of sterol biosynthesis, successive removal of two methyl groups from the C4 carbon requires NAD(P)H steroid dehydrogenase-like protein (NSDHL, ERG26 in yeast) and sterol 3-ketoreductase (yeast ERG27). NSDHL/ERG26 is found in the mammalian but not the yeast droplet proteome. GFP-NSDHL localizes to ER and lipid droplets in COS7 cells (37). Localization to both compartments depends on a C-terminal RKDK sequence; deletion of this sequence results in a Golgi pattern of fluorescence, suggesting that ER retention is a step in droplet trafficking. In NIH 3T3 cells transfected with GFP-NSDHL fusions, however, droplet localization is not obvious (38).

Whereas NSDHL/ERG26 localizes to droplets only in mammals, sterol 3-ketoreductase/ERG27 is found in the droplet proteomes of both mammals and fungi. In yeast, an ERG27-GFP is clearly localized to both droplets and ER (14). The mammalian ERG27 enzyme also has 17β-hydroxysteroid activity and has been termed 17HSD type 7; details of its distributions will be discussed below. Interestingly yeast ERG27 is required for ERG7 activity and stability, coimmunoprecipates with ERG7, and may facilitate its association with droplets (39).

ERG6 is specific to the yeast ergosterol pathway and has no mammalian ortholog. The protein is found exclusively in droplets and often serves as a marker for that compartment (14). If overexpressed, the protein accumulates in the ER (K. M. Szymanski and J. M. Goodman, unpublished observations).

Thus, several enzymes in cholesterol biosynthesis common to yeast and mammals are localized to droplets and to the ER to varying extents. Droplet localization can result in inactivation (ERG1) or transactivation (ERG7).

Regarding a role for droplets in steroid metabolism, two 17β-hydroxysteroid dehydrogenases (17HSDs) have been localized to mammalian droplets. One enzyme, 17HSD type 7 [now known to be identical to ERG27 (38)] can, in collaboration with aromatase, synthesize estradiol from androstenedione. It is found in droplets from 3T3 L1 adipocytes (basal and induced with adrenergic agonist) and CHO cells (23, 24); however, N-terminally GFP-tagged human and mouse proteins localized to the ER in Hela and NIH 3T3 cells (a C-terminally tagged protein was cytosolic) (38); effects of oleate addition were not tested. Nevertheless, synthesis of estrogen from adipose depots in postmenopausal women has been long established, and the association of 17HSD type 7 with droplets is suggestive of an involvement of this organelle in estrogen biosynthesis. The 17HSD type 7 enzyme has multiple transmembrane domains and thus may not be disposed on the droplet leaflet itself but instead on associated ER.

17HSD type 11 was identified in the droplet proteome of CHO K2 cells (24, 40). This form of 17HSD is active in testosterone metabolism in steroidogenic cells, catalyzing the conversion of 5α-androstanediol to 5α-androsterone (41). 17HSD type 11 can be induced by peroxisome proliferator-activated receptor alpha agonists (42). When CHO cells expressing the mouse type 11 enzyme fused to GFP are incubated with oleic acid, the protein redistributes from the ER to the periphery of droplets (43); the targeting domain for droplet localization was determined to be an N-terminal hydrophobic sequence followed by a small PAT-like domain (44). However, the protein is predicted

to have one to two particularly strong transmembrane domains based on computer-assisted analysis of the primary sequence; thus, it is unclear how localization on droplets per se can occur. This prediction further suggests the presence of a discrete site on the ER adjacent to droplets to which 17HSD type 11 may localize.

What is the need for dual localization of multiple sterol synthesizing enzymes to the bulk ER and droplets? Perhaps droplets (or specific ER sites next to droplets) can sequester these enzymes away from the biosynthetic machinery elsewhere in the ER (14). Thus, when sterol esters in the droplet are high, this mechanism would shut down further synthesis.

## TRIGLYCERIDE BIOSYNTHESIS

Microsomal enzymes traditionally are thought to catalyze triglyceride biosynthesis; however, in yeast, a major fraction of three enzymes that catalyze steps in triacylglycerol (TAG) formation are found largely in droplets, to a lesser extent in the ER. AYR1, which reduces acyldihydroxyacetone phosphate to acyl-glycerol-3-phosphate, and SLC1, which adds the second fatty acid to generate phosphatidic acid, are both largely in droplets. The major phosphatidic hydrolase, generating diacylglycerol (DAG), is largely soluble with a small amount in particulate fractions that probably represents localized to the ER (45). Finally, a fraction of DGA1 generating the final product, triacylglycerol, is localized to droplets (14). The mammalian ortholog (DGAT2) is the single enzyme in the pathway that is found in the vicinity of lipid droplet in mammals (46). DGAT2 is important for both de novo synthesis of TAG as well as remodeling of fatty acids on TAG (47); however, this enzyme is predicted to contain two membrane spans (48), so it may not be in the droplet monolayer but instead the associated ER. Enzymes in the TAG pathway (including DGAT2) are not found in droplet proteomes from mammalian sources.

## LIPOLYSIS AND PAT FAMILY PROTEINS

Lipases, hydrolases that cleave fatty acids from triglycerides and steryl esters, are common elements of droplet proteomes. Lipolysis is important not only for providing metabolic energy through oxidation pathways (intracellular or at distant targets) but also for production of signaling lipids such as eicosanoids.

Lipases can be constitutively active or tightly regulated. To gain access to substrate, they generally have to interact with members of the PAT family of proteins, which are thought to provide a protective coat around the droplet (49). The activation of hormone-sensitive lipase (HSL) in adipose tissue is the prototypic example of such an interaction (50, 51). HSL normally resides in the cytosol; however, upon adrenergic activation, protein kinase A (PKA) phosphorylates HSL. Concommitantly, PKA phosphorylates perilipin at multiple sites, which facilitates the binding of HSL to perilipin, a necessary step in HSL-catalyzed lipolysis. Phosphorylation of peripilin at serine 492 appears to mimic chronic adrenergic stimulation of this pathway, which causes the fragmentation of droplets into smaller particles, increasing surface area and facilitating lipolysis of triglycerides (52).

The knockout of HSL in mice was expected to cause an obese phenotype but did not (53), indicating that the action of one or more unknown lipase must be responsible for maintaining droplet volume. This result led to the identification of adipocyte triglyceride lipase (ATGL) by several groups, another commonly detected protein in droplet proteomes (54–56). Comparison of enzyme kinetics of different substrates led to the current model whereby ATGL first hydrolyzes triglycerides to DAG, whereas HSL further oxidizes DAG to monoacylglycerol (MAG) (57).

ATGL requires CGI-58, another protein commonly detected in the droplet proteome, for full activity (58). CGI-58 normally resides on droplets; however, upon phosphorylation by PKA, CGI-58 is released and can then recruit ATGL from the cytosol to droplets. ATGL mRNA is reduced by insulin, allowing net storage of triglycerides in the fed state (57).

In *S. cerevisiae*, there are five TGL (triglyceride lipase) genes. In growing cells, lipase activity is provided almost exclusively by TGL3 and TGL4 (59). TGL4 has a patatin domain as does mammalian ATGL, and mouse ATGL can partially complement the lipase deficiency of a *tgl4Δ* strain (59). A Tgl4-GFP construct localizes exclusively to droplets (60), unlike most droplet proteins that also localize to ER (www.yeastgenome.org). Tgl4p has recently been shown to be regulated by phosphorylation by the Cdk1/Cdc28 kinase (61). Activation by this kinase stimulates Tgl4 to provide precursors for phospholipid synthesis, which are essential for bud formation in the G1 phase of the cell cycle (61).

Yeast has three steryl esterases: TGL1, YEH1, and YEH2 (62). TGL1 was detected in the yeast droplet proteome (29). YEH1 and YEH2 were identified with a similar lipase region and shown to have steryl esterase activity. Although GFP-tagged TGL1 and YEH1, chromosomally expressed from the endogenous promoters, target to droplets, YEH2 targets to the plasma membrane (62). Whereas both Yeh1p and Tgl1p behave as integral membrane proteins, Yeh1p has a long hydrophobic region with both ends facing the cytosol, suggesting it does not interact with the core of the droplet. Surprisingly, Tgl1p appears to have a single membrane spanning domain with an (GFP-tagged) N terminus protected from the cytosol (62). Although this end may in fact interact with the core, it seems much more likely that the sequence exists in an aqueous environment, such as in a droplet-associated region of the ER.

Members of the PAT family, mentioned above, are often identified in proteomic studies of mammalian droplets, although orthologs exist in organisms as simple as insects (63, 64). PAT is named after the first three identified members of the family: perilipin, adipocyte differentiation-related protein (ADRP), and tail-interacting protein of 47 kDa (TIP47). The identification of perilipin in adipose tissue by Greenberg et al. (65) opened the door to

the idea that droplets are more than aggregated lipids. The reader is referred to recent reviews on PAT family proteins for a detailed discussion of their structure, expression patterns, and function (3, 49). The following discussion is limited to the role of PAT proteins in droplet metabolism.

PAT proteins are the most abundant proteins of the droplet. As such, one of their roles is to prevent adventitious lipolysis, perhaps by physically blocking association with lipases. Upon adrenergic stimulation of adipocytes, however, perilipin becomes phosphorylated, which both attracts HSL to the droplet surface, as previously described, and releases CGI-58 for binding and recruitment of ATGL to the surface. The metabolic roles of the other PAT family members, other than preventing binding of lipases, are less clear. ADRP and TiP47 are expressed in many tissues, whereas OXPAT and S3-12, other members of the PAT family, have narrower expression profiles (49).

Caveolin, also a member of many droplet proteomes, can traffic sterol and fatty acids to the droplet surface and may bridge perilipin and PKA, promoting lipolysis by HSL (3).

## TWO OTHER ENZYMES OF LIPID METABOLISM OF THE DROPLET PROTEOME AND A PROTEIN OF MYSTERY

The alcohol dehydrogenase ALDH3B1 is a common constituent of mammalian droplet proteomes. Recent data suggest that this enzyme reacts with medium- and long-chain aliphatic aldehydes, potential products of fatty acids released by the droplet, and thereby protects the cell from oxidative stress (66).

In yeast, a constitutent of the droplet is EHT1 [identified in a proteomics study initially as YBR177c (29)], an ethanol hexanoyl transferase, which produces medium-chain fatty acid ethyl esters (67). The function of these products in yeast is not known.

DHRS1 (short-chain dehydrogenase/reductase family member 1) has been localized to droplets in several studies and also to several other organelles (EntrezGene). The protein is likely an oxidoreductase based on sequence similarity but its precise function, if any, in the droplet is not known.

## THE STATE OF THE FIELD

Now that proteomics have revealed constellations of metabolic proteins associated with droplets in several species, research will shift toward several remaining puzzles: What directs proteins to droplets and what is the common element of these targeting sequences? What receptor system recognizes these sequences? Why are so many proteins dually localized, especially between droplets and ER? Is activity regulated by this partitioning? When are droplets connected to the ER, how are they connected (proteinaceous connections? continuous membrane?) and what regulates association and dissociation? As often occurs, structure (in this case, organellar structure) and function (metabolism) seem inextricably linked; both will likely have to be studied together.

The author regrets the lack of a comprehensive set of references but the limited length of this article forces the omission of many significant contributions to this field.

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