Minireview: Lipid Droplets in Lipogenesis and Lipolysis

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Organisms store energy for later use during times of nutrient scarcity. Excess energy is stored as triacylglycerol in lipid droplets during lipogenesis. When energy is required, the stored triacylglycerol is hydrolyzed via activation of lipolytic pathways. The coordination of lipid storage and utilization is regulated by the perilipin family of lipid droplet coat proteins [perilipin, adipophilin/adipocyte differentiation-related protein (ADRP), S3-12, tail-interacting protein of 47 kilodaltons (TIP47), and myocardial lipid droplet protein (MLDP)/oxidative tissues-enriched PAT protein (OXPAT)/lipid storage droplet protein 5 (LSDP5)]. Lipid droplets are dynamic and heterogeneous in size, location, and protein content. The proteins that coat lipid droplets change during lipid droplet biogenesis and are dependent upon multiple factors, including tissue-specific expression and metabolic state (basal *vs.* lipo-genic *vs.* lipolytic). New data suggest that proteins previously implicated in vesicle trafficking, including Rabs, soluble *N*-ethylmaleimide sensitive factor attachment protein receptors (SNAREs), and motor and cytoskeletal proteins, likely orchestrate the movement and fusion of lipid droplets. Thus, rather than inert cytoplasmic inclusions, lipid droplets are now appreciated as dynamic organelles that are critical for management of cellular lipid stores. That much remains to be discovered is suggested by the recent identification of a novel lipase [adipocyte triglyceride lipase (ATGL)] and lipase regulator [Comparative Gene Identification-58 (CGI-58)], which has led to reconsideration of the decades-old model of lipolysis. Future discovery likely will be driven by the exploitation of model organisms and by human genetic studies. (*Endocrinology* 149: 942–949, 2008)

O RGANISMS STORE LIPID when they take in more energy that can be immediately used. This excess energy is packaged and stored for later use when the need for energy outstrips available nutrient supply. In mammals, adipose tissue, in addition to its role as an endocrine organ, is specialized for storage and retrieval of energy in the form of triacylglycerol (TAG), but all eukaryotic cells, and even prokaryotes, are able to store limited amounts of lipid intracellularly in structures most commonly referred to as lipid droplets. Other terms for these structures include lipid bodies and adiposomes in animals and oil bodies in plants. Lipid droplets are structurally similar to circulating lipoproteins in that they have a core of esterified lipids (TAG, cholesterol ester, retinol esters, or ether lipids, depending on the cell type) that is encased by a phospholipid monolayer and a coat of specific proteins. During times of energy scarcity, the organism accesses these stores and retrieves the stored energy via the activity of lipid hydrolases, known as lipases. Increasingly, lipid droplets are being recognized as dynamic organelles that are regulated by evolutionarily conserved families of proteins, including the perilipin family. Review articles have summarized recent discoveries in the field (1–3). We will provide an overview of neutral lipid packaging through the generation of lipid droplets and of unpacking via lipolysis, with special focus on recent work that relates to lipid droplet assembly, enlargement, and movement.

Cellular Roles of Lipid Droplets

The sequestration of lipid in droplets provides a depot of stored energy that can be accessed in a regulated fashion according to metabolic need. The stored lipid can also be used as substrate for synthesis of other important cellular molecules, such as membrane phospholipids and eicosanoids. The products of TAG hydrolysis, diacylglycerol (DAG) and free fatty acids, may influence cell signaling either directly or via subsequent metabolism, for example to fatty acyl coenzyme A. Free fatty acids may influence gene expression by acting as ligands for nuclear receptors, such as the peroxisome-proliferator activated receptor (PPAR) family. Excess intracellular free fatty acids can disrupt phospholipid bilayer membrane integrity, alter lipid signaling pathways, and induce apoptosis (4). The esterification of free fatty acids with glycerol and the packing of TAG into coated droplets thus provide cells a means of regulating the availability of substrates for energy utilization and of lipid signaling molecules as well as of potentially toxic metabolites.

Over the past decade, significant attention has been paid to the association of type 2 diabetes with excess intracellular lipid in nonadipose tissues. Data from different labs have suggested various mechanisms by which intracellular lipids may disrupt cellular function in insulin-secreting cells (β-cells) and insulin-responsive cells (myocytes, cardiomyocytes, and hepatocytes). Incubation of several cell types with long-chain saturated fatty acids leads to increased *de novo* ceramide synthesis and increased production of reactive ox-

ygen species as well as activation of apoptosis (5, 6). Whether increased reactive oxygen species or ceramide is the primary trigger for apoptosis remains controversial and may depend on cell type. In skeletal muscle and liver, fatty acid metabolites such as DAG or fatty acyl coenzyme A activate atypical protein kinase C isoforms, thereby leading to serine/threonine phosphorylation and inhibition of insulin-responsive substrate proteins (reviewed in Ref. 7). Accumulation of lipids in peripheral tissues can be prevented by efficient sequestration of TAG in adipocytes. In this regard, it has been proposed that a major function of the adipocyte-secreted hormone leptin is to prevent ectopic lipid accumulation in nonadipocytes at least in part by promoting fatty acid oxidation and preventing induction of lipogenesis in muscle and liver (8). The storage of lipid in nonadipocytes in and of itself is not the likely culprit in lipotoxicity. In Chinese hamster ovary cells, coadministration of the monounsaturated fatty acid oleate with the saturated fatty acid palmitate increases the incorporation of palmitate into TAG and provides protection against palmitate-induced lipotoxicity (9). Thus, the sequestration of TAG into lipid droplets may protect against lipotoxicity. The case of endurance athletes is another example. Insulin-sensitive endurance athletes and insulin-resistant subjects with type 2 diabetes both have increased intramyocellular lipid (10), but the athletes also have increased capacity to oxidize fatty acids, thereby limiting accumulation of fatty acid metabolites (recently reviewed by Moro *et al.* in Ref. 11). Understanding the mechanisms responsible for packing and unpacking TAG in different tissues is, therefore, critical for designing strategies to protect cells from lipotoxicity.

The packaging of lipid into discrete storage droplets may help transport the lipid cargo to specific cellular destinations or direct the neutral lipids and their metabolites to specific metabolic or signaling pathways. Such coordination of lipid droplet metabolism is likely controlled by the lipid droplet coat proteins of the perilipin family, which include the founding member perilipin, as well as adipophilin [also known as adipocyte differentiation-related protein (ADRP or ADFP)], tail-interacting protein of 47 kilodaltons (TIP47), S3-12, and oxidative tissues-enriched PAT protein (OXPAT). This family has also been referred to as the PAT family in recognition of the first three members to be localized to lipid droplets, perilipin, adipophilin/ADRP, and TIP47 (12). These perilipin family proteins share sequence similarity and localize to lipid droplets, either constitutively (perilipin, adipophilin/ADRP) or in response to lipogenic and/or lipolytic stimuli (TIP47, S3-12, and OXPAT). For more comprehensive discussions of the PAT family proteins, the reader is referred to recent reviews (1, 3, 13).

In addition to the above noted functions for lipid droplets, recent studies have suggested unexpected roles as well. In addition to being a storage depot for lipid, lipid droplets may sequester specific proteins when levels of those proteins are high. Welte (14) has proposed that such proteins be termed refugee proteins, because lipid droplets provide them temporary shelter when they are not needed or desirable in the cellular compartment where they normally function. The mechanisms, regulation, and biological significance of protein sequestration on lipid droplets remain to be established,

but some intriguing data are emerging. For example, in the *Drosophila* egg, excess maternally derived histones are sequestered on lipid droplets and then move to the nucleus as the embryo develops (15). In this way, the early embryo may be protected from toxic effects of excess free histones and also have an accessible supply of histones for mobilization when needed later in development.

Lipid Droplet Biogenesis

According to the prevailing model, lipid droplets emerge from the endoplasmic reticulum (ER) lipid bilayer or from a subset of ER membranes as a lens of neutral lipid that then buds off from the cytoplasmic face of the bilayer to form a discrete nascent droplet within the cytoplasm (as reviewed in Refs. 1, 3, and 16). This model is attractive because it explains the origin of the phospholipid monolayer that surrounds lipid droplets and also localizes the birthplace of lipid droplets to the organelle with which enzymes of neutral lipid synthesis fractionate biochemically. That lipid droplets are surrounded by a phospholipid monolayer has been confirmed by cryoelectron microscopy (17), but this same study also found that the fatty acid composition of lipid droplet phospholipids differed from that of rough ER phospholipids. Ultrastructural studies have revealed intimate associations between lipid droplets and ER-like cisternal structures (18–20), and proteomic studies of lipid droplets isolated from various cell types, including 3T3-L1 adipocytes (21), Chinese hamster ovary cells (22), and a monocytic cell line (20), have revealed the presence of ER proteins. Points of apparent membrane continuity between ER-like structures and lipid droplets have been observed in some (18) but not all (19) studies. Incubation of 3T3-L1 adipocytes in oleate-rich medium results in the emergence of nascent lipid droplets that have a protein coat distinct from that of the preexisting perilipin-coated droplets (23). Transmission electron micrographs of oleate-loaded adipocytes reveal ER-like cisternae in close proximity to the surfaces of larger lipid droplets but not of the smallest, presumably nascent droplets (24). It has been pointed out that electron microscopy studies have not documented budding of nascent lipid droplets from the ER cytoplasmic leaflet (19). Such negative data do not refute the prevailing model but have suggested an alternative model, whereby the association of lipid droplets with ER-like membranes, like an egg cup (the ER) holding an egg (the lipid droplet) (19), does not reflect the site of lipid droplet origin but rather a means of lipid droplet expansion through transfer of lipid and adipophilin/ADRP. Thus, major fundamental questions regarding the biogenesis of lipid droplets remain to be answered.

Lipid Droplet Heterogeneity

The formation and maturation of lipid droplets and the involvement of perilipin family members in these processes have been most extensively studied in cultured adipocytes. Under resting conditions, the majority of adipocyte lipid droplets are coated by perilipin. However, when the cells are incubated with long-chain fatty acids, a new pool of smaller lipid droplets coated with S3-12 and TIP47, but not perilipin, emerges at the periphery of the cell (23, 24). Over time, more

centrally located, larger droplets acquire adipophilin/ADRP on their coats, in addition to TIP47 and S3-12. Large, centrally located droplets are coated by perilipin. At the interface of the adipophilin/ADRP- and perilipin-coated droplets, a subset of droplets is coated with multiple perilipin family protein combinations, such as adipophilin/ADRP or S3-12 in addition to perilipin (reviewed in Ref. 3). The heterogeneity of the lipid droplet coat during lipid loading of adipocytes suggests a coordinated process of lipid droplet maturation and movement from peripheral sites of synthesis to perinuclear sites of storage.

A fifth perilipin family member has been characterized independently by three groups and named myocardial lipid droplet protein (MLDP) (25), OXPAT (26), and lipid storage droplet protein 5 (LSDP5) (27). In COS cells that stably or transiently express OXPAT, OXPAT moves to lipid droplets in response to an influx of fatty acid (26, 27), similar to TIP47 and S3-12 in adipocytes. OXPAT transiently expressed in a mouse Leydig tumor cell line (25) and endogenous OXPAT in mouse primary cardiomyocytes (26) localize to lipid droplets in the absence of increased exogenous fatty acid.

Heterogeneity of lipid droplet coats has also been revealed in other experimental systems. Subcellular localization studies of proteins identified in a study of the *Drosophila* lipid droplet subproteome showed that specific proteins coat subsets of lipid droplets within the same cell (28). The authors concluded that the proteins that coat individual lipid droplets may constitute a zip code for lipid droplets that perform different functions within the cell. In another example of lipid droplet heterogeneity, lipid droplets that accumulate in term fetal membranes during gestation are coated with different perilipin family members depending on lipid droplet size and cell type (29).

The heterogeneity of lipid droplets with respect to size, location, and associated proteins within a given cell or tissue and between different tissues suggests that subpopulations of lipid droplets likely have specialized functions in lipid storage and metabolism.

Potential Functions of Perilipin Family Members

That each perilipin family member has a specialized function is strongly suggested by their differential expression in mouse tissues (26), in addition to their differential localization and behavior in cells such as adipocytes. Adding to this complexity, some perilipin family members are expressed as tissue-specific isoforms generated by alternative splicing; the best studied example is perilipin itself (30). All perilipin isoforms are present in cells that make steroid hormones; the perilipin A and B isoforms are also expressed in adipocytes. Perilipins have also been detected in macrophages and smooth muscle cells of human atheroma (31). Adipophilin/ADRP and TIP47 are expressed in most if not all cell types, although little adipophilin/ADRP protein is detectable in mature adipocytes. S3-12 expression is confined largely to white adipose tissue but is detectable in heart and skeletal muscle (23, 32, 33). OXPAT is found in tissues that have high rates of fatty acid oxidation, such as heart, brown adipose tissue, fasted liver, and skeletal muscle, especially muscle with predominantly slow-twitch fiber types (25–27).

Most functional data for the family have come from studies of perilipin itself. Perilipin was initially identified as the major adipocyte protein phosphorylated in response to activation of protein kinase A (PKA) (34). Its localization surrounding neutral lipid storage droplets in adipocytes led to the notion that it forms a hormonally regulated barrier between cytosolic lipases and the neutral lipids within. Consistent with this model, heterologous expression of perilipin A in 3T3-L1 preadipocytes leads to increased TAG storage by reducing the rate of TAG hydrolysis rather than by promoting TAG synthesis (35). This model underwent dramatic revision when two groups independently reported the metabolic phenotype of perilipin knockout mice (36, 37). The previously hypothesized barrier function of perilipin was confirmed by the findings that these mice were lean and protected from genetic and diet-induced obesity due to increased basal TAG breakdown. However, an additional role in the control of cellular lipid stores was suggested by the observation that hormone-stimulated lipolysis in these mice was reduced. Thus, in addition to keeping lipases at bay under basal conditions, perilipin appears to coordinate the recruitment and/or activation of lipases under lipolytic conditions, as discussed below.

Tansey and colleagues (37) noted that adipophilin/ADRP protein was increased in the adipose tissue of perilipin knockout mice and that adipophilin/ADRP coated adipocyte lipid droplets in lieu of perilipin. Thus, adipophilin/ADRP is able to replace perilipin on the lipid droplet surface when perilipin is absent, but it cannot replace perilipin functionally to confer equivalent protection from basal lipolytic mechanisms or to confer full catecholamine-induced lipolysis. In fibroblasts (38), hepatic stellate cells (39), and macrophages (40, 41), overexpression of adipophilin/ADRP promotes accumulation of neutral lipid and/or lipid droplets. Conversely, knockdown of adipophilin/ADRP in macrophages using small interfering RNA (siRNA) dramatically reduces lipid accumulation and lipid droplet size and number (41). A straightforward explanation of these data would be that, like perilipin, adipophilin/ADRP shields TAG stores from cytosolic lipases, although less effectively. This hypothesis has been challenged by the observation that adipophilin/ADRP overexpression in macrophages does not appear to protect TAG stores from lipolysis (41). Consistent with the *in vitro* studies, knockout of adipophilin/ADRP in mice has a phenotype of reduced TAG accumulation in the liver and reduced hepatic steatosis in response to high-fat feeding (42). The reduction in hepatic TAG is not explained by significant changes in hepatic fatty acid synthesis or uptake, TAG production, very-low-density lipoprotein secretion, or β-oxidation. In contrast to reduced hepatic cytosolic TAG, adipophilin/ADRP knockout mice have a 2-fold increase in hepatic microsomal TAG, which has led to the hypothesis that absence of adipophilin/ADRP reduces partitioning of TAG into lipid droplets at the site of TAG synthesis, the ER (42). A recent report has shown that these adipophilin/ADRP knockout mice unexpectedly express an amino-terminal truncation of adipophilin/ADRP, termed Δ2,3-ADPH, that coats lipid droplets in mammary secretory epithelial cells after parturition (43). Whether Δ2,3-ADPH is expressed in other tissues of adipophilin/ADRP knockout mice is not

known. Thus, adipophilin/ADRP may play a more indispensable role in cellular lipid metabolism than determined thus far.

The functional role of TIP47 has been examined recently by siRNA loss-of-function studies (44). Sztalryd and colleagues (44) generated clonal embryonic fibroblast cell lines from adipophilin/ADRP knockout and wild-type mice. No differences in lipid droplet formation, fatty acid uptake, or lipolysis are detectable between these cell lines, perhaps due to the observed up-regulation of TIP47 in the knockout cells. However, siRNA knockdown of TIP47 in the adipophilin/ADRP knockout cells, but not wild-type cells, results in reduced lipid droplets, reduced incorporation of oleate into TAG, and increased oleate incorporation into phospholipids.

The genes for TIP47, S3-12, and OXPAT reside within 200 kb on mouse chromosome 17 (26, 27). The *s3-12* gene is immediately downstream from the *oxpat* gene. Despite this proximity, the expression of S3-12 and OXPAT in mouse tissue is reciprocal with S3-12 being expressed primarily in the tissue specialized for lipid storage, white adipose tissue, and OXPAT being expressed in tissues with a high capacity for lipid utilization, specifically heart, skeletal muscle, brown adipose tissue, and liver. Fasting induces OXPAT protein in liver (26, 27) and heart (25, 27). Ectopic expression of OXPAT promotes both β-oxidation of long-chain fatty acids and TAG accumulation (26), which is similar to the phenotype of endurance trained athletes who both store and burn more fat in skeletal muscle. The reciprocal patterns of expression of S3-12 and OXPAT suggest that they have reciprocal functions with respect to cellular lipid metabolism, but this notion remains for experimental validation.

Trafficking and Lipid Droplets

Live-cell microscopy of 3T3-L1 adipocytes has revealed temporal and spatial changes in lipid droplets both during adipocyte differentiation (45) and during oleate loading (24). The realization that lipid droplets are dynamic organelles suggests that movement of the droplet itself and of constituent components to and from the droplet must be highly regulated. This regulation appears to be orchestrated by proteins previously associated with vesicular trafficking pathways such as Rabs, soluble *N*-ethylmaleimide sensitive factor attachment protein receptors (SNAREs), motor proteins, and cytoskeletal components.

Endocytic trafficking pathways in cells are often associated with one or more Rab proteins, which are cycling GTPases that serve as functional addresses for vesicles. A plethora of Rab proteins, at least 18 to date, have been associated with lipid droplets on the basis of proteomic studies of lipid droplets isolated by gradient fractionation (15, 21, 22, 46). In most cases, localization of specific Rab proteins to lipid droplets has not been confirmed morphologically or functionally; however, such data are beginning to appear. Ozeki and colleagues (47) identified 11 different Rabs by proteomic analysis of lipid droplets from HepG2 cells, but only Rab18 showed conclusive and consistent labeling of lipid droplets by immunofluorescence microscopy of cells transfected with tagged Rab cDNAs. Localization of Rab18 to lipid droplets was dependent on its functional status in that wild-type and a constitutively GTP-bound Rab18 mutant (Q67L) associated with lipid droplets but a constitutively GDP-bound Rab18 mutant (S22N) did not. In HepG2 cells, overexpression of Rab18 was associated with increased association of lipid droplets with membrane cisternae that were often continuous with the rough ER. Functional relevance of these findings to lipid metabolism has been strongly suggested by the finding that Rab18 association with lipid droplets increases upon lipolytic stimulation of cells (48). Rab18 may facilitate lipid droplet association with the ER to promote lipid transfer between these compartments (2, 47).

Other Rab GTPases have been functionally implicated in lipid droplet biology. In addition to Rab18, Liu and colleagues (49) observed Rab5 or Rab11 on the surface of isolated lipid droplets by immunogold electron microscopy. In cell-free experiments, Rab5 and Rab11 were recruited to lipid droplets in a GTP-dependent manner and were extractable from lipid droplets by Rab guanine diphosphate dissociation inhibitor. Rab5, in particular, may mediate recruitment of early endosome antigen 1-positive early endosomes to lipid droplets (50).

The parallels between neutral lipid-cored lipid droplet trafficking and aqueous-cored vesicle trafficking have been discussed (3). SNARE proteins on vesicles and target membranes are used in fusion events between aqueous-cored vesicles and target membranes (51, 52). Recent data from Boström and colleagues (53) suggest that SNARE proteins are involved in the fusion of lipid droplets with one another. These investigators found that multiple known SNARE complex proteins, including synaptosomal-associated 23-kDa protein (SNAP23), SNAP25, syntaxin-5, N-ethylmaleimide-sensitive factor, α-SNAP, and vesicle-associated membrane protein 4, coprecipitated with histidine-tagged adipophilin/ADRP. These results were confirmed for all but SNAP25 either biochemically by lipid droplet fractionation or morphologically by immunoelectron microscopy. The siRNA-mediated knockdown of SNAP23, syntaxin-5, or vesicle-associated membrane protein 4 resulted in a decrease in lipid droplet fusion events and in lipid droplet size but not in reduced TAG accumulation. Noting that SNAP23 is also required for fusion of glucose transporter 4 (GLUT4)-containing vesicles with each other and with the plasma membrane in insulin-responsive cells, Boström and colleagues (53) investigated the relationship between lipid droplet-associated SNAP23 and insulin-stimulated glucose transport in HL-1 cells, a cardiomyocyte cell line. They found that oleate loading of these cells led to movement of SNAP23 from the plasma membrane to intracellular sites, including lipid droplets. The oleate-induced reduction in plasma membrane SNAP23 was associated with reduced insulin-stimulated glucose uptake and plasma membrane-bound GLUT4, effects that were prevented by concomitant expression of a SNAP23-cyan fluorescent protein fusion protein. These results suggest a novel mechanism to explain the association of ectopic lipid accumulation with peripheral insulin resistance, at least in myocytes: competition between two cellular trafficking processes, lipid droplet fusion, and GLUT4 vesicle fusion with the plasma membrane, for a limiting amount of a SNARE protein, a competition that lipid droplets appear to win.

Lipid droplet fusion requires elements of the cytoskeleton in addition to SNARE proteins. Depolymerization of microtubules with nocodazole inhibits lipid droplet fusion (54). In flies, movement of lipid droplets along microtubules requires dynein, a microtubule minus end motor (55), which has been shown to co-immunoprecipitate with adipophilin/ADRP (54). Phosphorylation of dynein by ERK2 enhances the association of dynein with lipid droplets (56). Inhibition of dynein either chemically with vanadate (54) or by neutralizing antibody (56) decreases lipid droplet formation. Taken together, these results suggest that lipid droplets move along microtubules in a dynein-dependent manner.

The recent data summarized in this section suggest that lipid-cored droplets use cellular machinery similar to that used by aqueous-cored vesicles to move to targeted cellular locations and to fuse with each other. Future work will exploit these similarities to provide additional insights into lipid droplet biology based upon the extensive literature that cell biologists have generated in the field of vesicle and protein trafficking.

Fat Mobilization from Lipid Droplets

During times of nutrient scarcity, TAG stored within lipid droplets is catabolized into free fatty acids and glycerol in a process known as lipolysis. The glycerol and free fatty acids liberated from adipocyte lipid droplets enter the circulation. Glycerol and fatty acids are substrates for gluconeogenesis and ketogenesis, respectively, in the liver. Skeletal muscle and the heart use fatty acids for energy provision via mitochondrial β-oxidation and the generation of ATP. Although adipocyte lipolysis has been the primary focus of research effort, all tissues and cell types must be able to release free fatty acids from the TAG stored in lipid droplets. Lipolysis has been the subject of several excellent reviews (57–60). In the best-characterized lipolytic pathway, catecholamine binding to β-adrenergic G protein-coupled receptors on the plasma membrane generates a signaling cascade that activates cAMP-dependent PKA. The anabolic hormone insulin inhibits lipolysis by stimulating a phosphodiesterase that breaks down cAMP. PKA activation ultimately leads to the hydrolysis of a fatty acids from TAG to yield DAG, from DAG to yield monoacylglycerol, and finally from monoacylglycerol to yield the glycerol backbone. The molecular events of this process have been under investigation for the past four decades.

Over the years, a model of lipolysis has been developed and widely accepted in which activation of PKA leads to phosphorylation of a cellular lipase known as hormone-sensitive lipase (HSL). In support of this model, lipolytic activation of cultured adipocytes is associated with movement of HSL from the cytosol to the surface of lipid droplets, where it acts on the neutral lipids within (61). Perilipin has emerged as a critical organizing component of these changes, and serine phosphorylation of perilipin on one or more of six PKA consensus sites is a key mediator of this organization. Mutational analyses of the consensus PKA sites of perilipin have implicated one or more of these sites in HSL docking to lipid droplets and in maximal lipolysis, as recently reviewed (1). During hormone-stimulated lipolysis in adipocytes, major rearrangements take place in the morphology of lipid droplets and in the distribution of lipid droplet-associated proteins. Activation of PKA over several hours results in fragmentation of lipid droplets, which greatly increases the surface area of droplets available for lipase action. This fragmentation is dependent upon phosphorylation of perilipin at serine 492 (62).

The model of HSL activation as the rate-limiting step in lipolysis of triacylglycerol was brought into question when three groups independently knocked out HSL in mice and surprisingly observed that the mice were not obese (63–65). Rather than accumulating TAG in tissues, HSL knockout mice accumulate DAG (65). These results led to the search for additional lipases responsible for TAG hydrolysis. In 2004, a novel TAG lipase was reported by three groups and given three different names: desnutrin (66), adipocyte triglyceride lipase (ATGL) (67), and phospholipase A2ζ (68). Following publication of these initial reports, ATGL has been intensively studied in mouse models and in humans, and its critical role in cellular lipid metabolism established, although its precise role is still being worked out. ATGL knockout mice have increased fat pad size and accumulate excess TAG in most tissues (69). These mice accumulate so much TAG in the heart that they die of congestive heart failure. Like HSL knockout mice, ATGL knockout mice demonstrate diminished mobilization of free fatty acids from adipose stores in response to catecholamines. It has been proposed that in the mouse, ATGL and HSL act coordinately such that ATGL is the primary lipase responsible for hydrolyzing the first fatty acid from TAG and that HSL is the primary DAG lipase (57). Whether this model applies to lipolysis in humans has been questioned (70). A competing model has been proposed according to which HSL is the primary TAG lipase responsible for catecholamine-stimulated lipolysis from adipocytes and ATGL is the primary TAG lipase for basal lipolysis (59). Regardless of which model may be correct, the importance of ATGL in human cellular lipid metabolism has been established by the identification of mutations in the human ATGL gene that are associated with a neutral lipid storage disease in which excess TAG accumulates in tissues such as muscle and leukocytes (71, 72).

In contrast to HSL, ATGL appears to reside on the lipid droplet surface independent of PKA activation (67). Rather than its activation being controlled by phosphorylation and translocation, ATGL is activated at least 20-fold by interaction with CGI-58, a member of an esterase/lipase family of proteins (73). Comparative Gene Identification-58 (CGI-58) is also known as α/β-hydrolase domain-containing protein 5 (Abhd5). CGI-58 lacks lipase activity itself but activates the lipase activity of ATGL likely via protein-protein interaction. Fluorescence resonance energy transfer studies support a model in which CGI-58 binds to perilipin in adipocytes under basal conditions but releases from perilipin upon lipolytic stimulation (74). The gene for CGI-58 is mutated in humans with Chanarin-Dorfman syndrome (CDS) (75), which is characterized by accumulation of TAG in tissues. Individuals with this syndrome phenotypically resemble those with ATGL mutations, except for the prominence in CDS of a skin condition known as ichthyosis. Notably, mutant CDS proteins do not activate ATGL activity (73).

As noted above, models for the molecular mechanisms of lipolysis have undergone significant revision over the past few years. Additional insights will come from the study of novel lipases and their regulators, especially those that remain to be discovered in nonadipocytes.

Conservation of Lipid Droplet Biology

The coating of lipid droplets by proteins and the roles of those proteins in lipid metabolism are conserved across species. Perilipin family proteins in *Drosophila* (LSD1 and LSD2) and *Dictyostelium* (LSD) target to lipid droplet surfaces when expressed in mammalian cells (12). *Drosophila* store energy as TAG in an organ known as the fat body. LSD2 deletion mutants are lean, whereas LSD2 overexpression mutants are obese (76). Lipid droplets in fly embryos undergo changes in their movement and localization during development; these changes are disrupted in embryos that lack LSD2 (77). Thus, LSD2 appears to provide a functional link between lipid droplet transport and lipid metabolism. Flies also express an ortholog of ATGL, the brummer lipase. Flies with loss-of-function mutations in brummer accumulate excess neutral lipids (78) similarly to ATGL knockout mice and humans with ATGL mutations. Lipid droplet proteins that regulate lipid stores also have been found in organisms as diverse as *Caenorhabditis elegans* (79) and yeast (80, 81).

Looking Forward

Intracellular lipid droplets have gone from being largely ignored as static cytoplasmic inclusions to being actively studied as dynamic organelles that regulate cellular lipid stores and serve as depots not only for fat but also for specific proteins. Studies in rodent models and in humans that have suggested toxicity from ectopic lipid accumulation (excess lipid in nonadipocytes) have reinforced the importance of understanding how lipid droplets develop and function. The pioneering work of Constantine Londos and his colleagues (18, 34, 35, 37) on perilipin in mammalian adipocytes has led to the identification of a complex set of interacting proteins that coordinately regulate lipid stores and lipid substrates in diverse cells in diverse organisms. The pace of discovery has increased as new technologies have been brought to bear and investigators from other fields have become interested in lipid droplet biology. Proteomic approaches have identified well over a hundred different proteins in isolated lipid droplet fractions, many of them not previously associated with lipid metabolism. Continuing work will establish the functional relevance of these putative lipid droplet proteins. The evolutionary conservation of the molecular mechanisms of lipid droplet formation and lipolysis has been exploited in high-throughput screens for genes that influence lipid accumulation in model organisms. Discoveries in yeast, *C. elegans*, and *Drosophila* can be investigated in mammalian systems.

Much remains unknown about lipid droplet biology in humans. Do variations in the human genes for lipid droplet proteins influence susceptibility to obesity or obesity-associated disorders such as nonalcoholic fatty liver disease, cardiomyopathy, insulin resistance/type 2 diabetes, or atherosclerosis? Single-nucleotide polymorphism and haplotype analyses of the human gene for perilipin (PLIN) have shown

associations with obesity risk in different ethnic groups (82–84). Homozygosity for one perilipin single-nucleotide polymorphism is associated with increased basal and catecholamine-stimulated lipolysis and decreased perilipin protein in sc adipocytes of obese women (85). Whether polymorphisms in the genes for other perilipin family members correlate with obesity or lipolytic rate is not known. Indeed, much remains to be determined about the specific functions of each of the perilipin family members. Much, no doubt, will be learned from gain- and loss-of-function studies in mice and from translational studies in humans.

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