thematic review

Thematic review series: Adipocyte Biology

The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis

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Abstract **The majority of eukaryotic cells synthesize neutral lipids and package them into cytosolic lipid droplets. In vertebrates, triacylglycerol-rich lipid droplets of adipocytes provide a major energy storage depot for the body, whereas cholesteryl ester-rich droplets of many other cells provide building materials for local membrane synthesis and repair. These lipid droplets are coated with one or more of five members of the perilipin family of proteins: adipophilin, TIP47, OXPAT/MLDP, S3-12, and perilipin. Members of this family share varying levels of sequence similarity, lipid droplet association, and functions in stabilizing lipid droplets. The most highly studied member of the family, perilipin, is the most abundant protein on the surfaces of adipocyte lipid droplets, and the major substrate for cAMP-dependent protein kinase [protein kinase A (PKA)] in lipolytically stimulated adipocytes. Perilipin serves important functions in the regulation of basal and hormonally stimulated lipolysis. Under basal conditions, perilipin restricts the access of cytosolic lipases to lipid droplets and thus promotes triacylglycerol storage. In times of energy deficit, perilipin is phosphorylated by PKA and facilitates maximal lipolysis by hormone-sensitive lipase and adipose triglyceride lipase. A model is discussed whereby perilipin serves as a dynamic scaffold to coordinate the access of enzymes to the lipid droplet in a manner that is responsive to the metabolic status of the adipocyte.**—Brasaemle, D. L. **The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis.** ***J. Lipid Res.*** **2007.** 48: **2547–2559.**

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The ability to synthesize sterol esters or triacylglycerols and store these neutral lipids in cytoplasmic lipid droplets is a universal property of eukaryotes from yeast to humans. Although the presence of lipid droplets in cells and tissues had been noted in early observations of stained histological sections, the elucidation of the functions and composition of lipid droplets is a relatively recent development. Early hypotheses that lipid droplets provide a homeostatic mechanism to regulate intracellular lipid levels date back to studies in the 1970s in the laboratory of Michael S. Brown and Joseph L. Goldstein showing the esterification of cholesterol derived from the receptor-mediated uptake of low density lipoproteins and the depletion of cholesteryl ester stores after the withdrawal of lipoproteins from the culture medium of cells (1–3). Despite early speculation that cytosolic lipid droplets provide an important source of cholesterol for the maintenance, repair, and synthesis of membranes, very few studies throughout the 1970s and 1980s addressed the formation or dissolution of lipid droplets.

The first hint that proteins coat the cytosolic surfaces of intracellular lipid droplets and play critical roles in regulating neutral lipid metabolism emerged with the discovery of perilipins on adipocyte lipid droplets in the laboratory of Constantine Londos in 1991 (4). Since the identification of perilipins, four additional lipid droplet proteins with sequence similarity to perilipins have been described in vertebrates, with two additional family members in insects; these proteins include adipophilin (also called adipose differentiation-related protein), TIP47 (for Tail-Interacting Protein of 47 kDa), S3-12, OXPAT (also called Myocardial Lipid Droplet Protein or MLDP and Lipid Storage Droplet Protein 5 or LSDP5), and LSD1 and LSD2 (for Lipid Storage Droplet proteins 1 and 2) in vertebrates and insects, respectively. These proteins constitute the most abundant structural proteins on lipid droplets. Recent proteomics analyses have identified numerous enzymes involved in lipid metabolism and membrane trafficking on lipid droplets isolated from cultured mammalian cells or tissues (5–10); investigations of the localization of various newly identified proteins in cultured cells have further defined the lipid

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Abbreviations: ATGL, adipose triglyceride lipase; MEF, mouse embryonic fibroblast; PKA, protein kinase A; TNF-α, tumor necrosis factor-α.

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droplet as a distinct subcellular compartment or organelle. In the past few years, interest in the biology of lipid droplets has increased rapidly (11); the lipid droplet is now recognized as a dynamic organelle with a distinct lipid and protein composition and important functions in the maintenance of cellular lipid homeostasis, energy storage, and the production of signaling lipids.

Lipid droplets in plants have classically been termed oil bodies. The study of proteins associated with oil bodies in the seeds of plants began in the 1970s and 1980s, with the first full-length sequence of an oleosin described in 1990 (12); these low molecular weight proteins are integral components of plant oil bodies without apparent homologs in vertebrates. Discussion of the biology of plant lipid droplets (13), as well as a rapidly growing literature on yeast lipid droplets (reviewed recently in Ref. 14), are beyond the scope of this discussion, which will focus on building a model for how perilipin and related structural lipid droplet-associated proteins function to regulate the metabolism of triacylglycerols.

LIPID CONTENT AND FUNCTIONS OF LIPID DROPLETS IN MAMMALIAN CELLS AND TISSUES

A cytosolic lipid droplet has a comparable micellar structure to a serum lipoprotein. In the majority of cells, the neutral lipid core contains triacylglycerols and cholesteryl esters; in some types of cells, the core may also store retinol esters or ether lipids of the monoalkyl or monoalkenyl diacylglycerol classes (15). The triacylglycerol-rich lipid droplets of adipocytes provide the largest storage depot for energy in the form of esterified fatty acids that are mobilized to skeletal muscle and other tissues of the body for metabolism through β-oxidation to support ATP production. Lipolysis of adipocyte triacylglycerol also releases glycerol that is transported to liver for metabolism by either gluconeogenesis or glycolysis. Many other types of cells have cholesteryl ester-enriched lipid droplets that provide a source of cholesterol for membrane synthesis and repair; additionally, steroidogenic cells of the adrenal cortex, testes, and ovaries used stored cholesteryl esters as a source of substrate for steroid hormone synthesis (16–19). Lipid droplets within the stellate cells of liver have a uniquely high content of retinol esters that can be mobilized for use by cells throughout the body (20). Specialized interstitial cells within the lung, termed lipofibroblasts, store triacylglycerols that are metabolized to provide substrate for the synthesis of surfactant phospholipids in type II alveolar epithelial cells (21). Additionally, secretory epithelial cells in the mammary gland extrude cytosolic triacylglycerol-rich lipid droplets across the apical membranes to supply the lipid component of milk (22). Finally, lipid droplets provide substrates for the synthesis of signaling lipids and mediators of inflammation in leukocytes and other types of cells (23).

The core lipid is surrounded by a phospholipid monolayer (24, 25) containing cholesterol into which specific proteins may be embedded or peripherally associated through electrostatic interactions. The phospholipid composition of lipid droplets from several types of cells has been characterized; phosphatidyl choline is the most abundant phospholipid (15, 25), followed by phosphatidyl ethanolamine, phosphatidyl inositol, ether-linked phosphatidyl choline and phosphatidyl ethanolamine, and lysophosphatidyl choline and lysophosphatidyl ethanolamine, with very low levels of sphingomyelin, phosphatidic acid, and phosphatidyl serine (15). Importantly, the relative content of specific phospholipids, as well as the acyl chain composition of the phospholipids on lipid droplets, differs from that of bulk membranes or microsomes; the acyl chains are more highly unsaturated (15, 25), suggesting potentially unique origins and functions of lipid droplet phospholipids.

THE PERILIPIN FAMILY OF STRUCTURAL LIPID DROPLET PROTEINS: LOCALIZATION TO LIPID DROPLETS

The lipid droplets of cells from vertebrates contain one or more of five related structural proteins: perilipin, adipophilin, TIP47, S3-12, and OXPAT/MLDP (**Fig. 1**). Collectively, these proteins have been referred to in the literature as the PAT family of proteins, named after Perilipin, Adipophilin, and TIP47. Adipophilin (26, 27) and TIP47 (28–30) are expressed in the majority of cells and share the highest identity (43%) between amino acid sequences. Whereas adipophilin localizes exclusively to lipid droplets (26, 27), TIP47 is stable both as a soluble cytosolic protein and when associated with lipid droplets (29–32). The crystal structure of the C-terminal 60% of TIP47 (33) reveals clues that may explain this dual localization: four amphipathic helices form a bundle in solution that is similar to the four helix bundle of the N terminus of lipid-free apolipoprotein E, an exchangeable apolipoprotein. Although lipid-free apolipoprotein E is stable in solution and circulates in the blood, biophysical studies show that the four helix bundle of apolipoprotein E opens during lipid binding to embed the hydrophobic surfaces of the helices into the acyl chains of the surface phospholipid monolayer of lipoproteins (34, 35). Similarly, TIP47 is rapidly recruited from the cytosol to nascent lipid droplets when cells are provided with fatty acid substrates for triacylglycerol synthesis and storage (30, 32); thus, opening of the four helix bundle may facilitate TIP47 binding to lipid droplets, which, in turn, may stabilize the newly formed lipid droplets (36).

Alignment of the adipophilin amino acid sequence with that of TIP47 shows significant sequence similarity (33), suggesting that the C terminus of adipophilin may also fold into a series of four amphipathic helices; however, adipophilin is highly unstable in the cytoplasm and is subject to rapid proteasome-mediated degradation (37–39). Thus, significant variation in the amino acid sequence of adipophilin may alter the structure to prevent closure of the four helix bundle and the dissociation of adipophilin from the surfaces of lipid droplets to fold into a stable soluble protein. Interestingly, published deletion mutagenesis

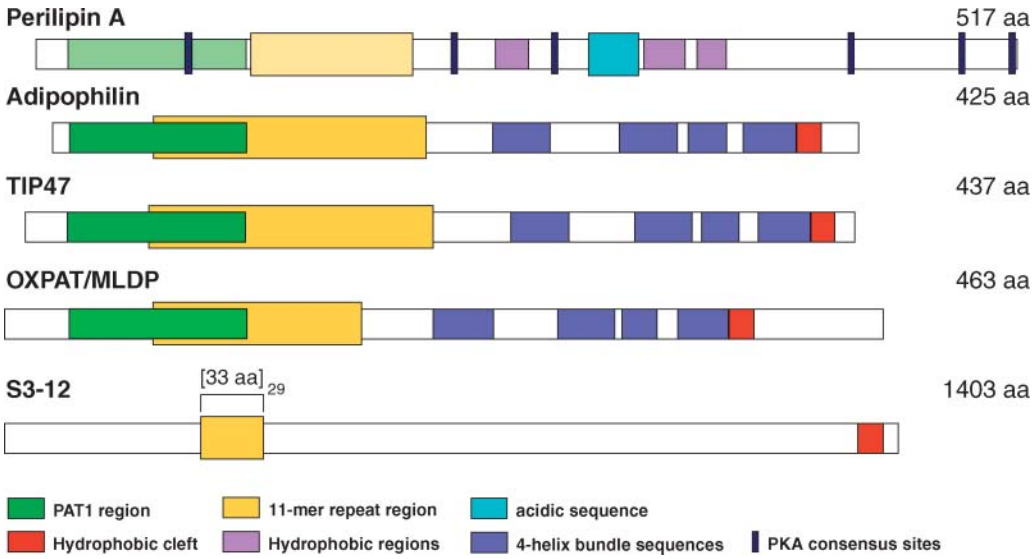

Fig. 1. Schematic diagram of the structural features of perilipin A and related proteins. The positions of structural features that are shared by the perilipin family of proteins are shown; greater intensity of color represents higher similarity of the sequences between family members, whereas lighter color represents reduced sequence similarity. Mouse sequences are depicted. The N termini of perilipin A, adipophilin, TIP47, and OXPAT/MLDP, but not S3-12, contain 100 amino acid (aa) sequences that are highly conserved between members of the family (green). Overlapping with these sequences are stretches of amino acids containing 11-mer repeat sequences that are predicted to fold into amphipathic helices (maize); definition of the exact boundaries of these helical sequences will require elucidation of the structures of the individual proteins. The most extensive section of 11-mer repeat sequences occurs in S3-12, which has at least 87 tandem 11-mer repeats (48). Following the putative 11-mer repeats, sequences of TIP47 fold into a four helix bundle of amphipathic α-helices (33) (blue); both adipophilin and OXPAT/MLDP share sequence similarity in this region. In contrast, within this region, perilipin has three sequences of moderate hydrophobicity (lilac) and a highly acidic sequence (cyan). The C termini of adipophilin, TIP47, OXPAT/MLDP, and S3-12, but not perilipin A, contain a highly conserved sequence of 14 amino acids that folds into a hydrophobic cleft in TIP47 (33) (red). Perilipin A is unique among members of the protein family in having six consensus sequences for the phosphorylation of serine residues by protein kinase A (PKA) (charcoal) dispersed throughout its primary amino acid sequence. (Illustration by Rick Hasney.)

studies for TIP47 (40) and adipophilin (41–43) reveal that sequences predicted to form these four helices are insufficient to direct the targeting of either nascent protein to lipid droplets. However, a natural splice variant of TIP47 mRNA produces a truncated form of the protein in human steroidogenic cells that includes the four helix bundle sequence but lacks the majority of N-terminal amino acids (44); this alternative form of TIP47 reportedly associates with lipid droplets (31).

The most recently described member of the perilipin family has been given several names, including OXPAT (for a PAT family protein expressed in oxidative tissues) (45), MLDP (46), and LSDP5 (47). OXPAT/MLDP is most highly expressed in heart and slow-twitch muscle, with lower levels in fast-twitch muscle, liver, white and brown adipose tissue, testis, and adrenal gland (45–47). OXPAT/MLDP is related to TIP47 (30% identity) and adipophilin (26% identity) throughout the amino acid sequence (45). Furthermore, like TIP47, OXPAT/MLDP is stable in the cytoplasm but is recruited to lipid droplets under conditions that promote lipid droplet formation (45).

The two remaining members of the protein family, perilipin and S3-12, have divergent amino acid sequences relative to TIP47, adipophilin, OXPAT/MLDP, and each other (Fig. 1). They also show limited tissue distribution. S3-12 expression is highest in white adipocytes, with very low expression in heart and skeletal muscle and little or no expression in brown adipocytes (48, 49). Perilipin expression is highest in white and brown adipocytes (4, 50–52), with lower levels of expression in steroidogenic cells (53) of the adrenal cortex, testes, and ovaries. The divergence of amino acid sequences and restricted tissue expression patterns suggest that perilipin and S3-12 have evolved for highly specialized functions specific to adipocytes and a few other types of cells.

Sequence similarity between perilipin and other members of the protein family is limited. The most highly conserved sequence includes ~100 amino acids located at the N terminus of the perilipin sequence and is conserved between perilipin, adipophilin, TIP47, and OXPAT/MLDP but not S3-12 (Fig. 1). This sequence is also similar to N-terminal sequences in two lipid droplet proteins expressed in insects, LSD1 and LSD2. The function of this well-conserved sequence has not yet been identified, and the sequence is dispensable for targeting of newly synthesized perilipin to lipid droplets (54, 55). Deletion mutagenesis studies have shown that three sequences of moderately hydrophobic amino acids located in the central portion

of the primary amino acid sequence are required to direct nascent perilipin to lipid droplets and to anchor the protein into the droplet (56); the structural conformation of these sequences and the mechanism that tethers perilipin to lipid droplets are unknown. Once bound to lipid droplets in adipocytes, perilipin has a half-life exceeding 70 h under basal (nonlipolytic) conditions (57) and is not readily released from isolated lipid droplets by altering the pH or the concentration of salt or nonionic detergents (54). These observations suggest that perilipin is a constitutive protein of lipid droplets and does not readily exchange into the cytoplasm.

Three protein isoforms of perilipin have been described that arise from the translation of alternatively spliced mRNA (50, 58). Perilipin A is the largest protein (517 amino acids in mice) and the most abundant protein on adipocyte lipid droplets (4, 50); perilipin B, a less abundant protein, shares 405 amino acids with perilipin A followed by 17 unique amino acids at the C terminus (in mice) (50, 58); perilipin C is an even shorter isoform that is expressed only in steroidogenic cells (53, 58). Importantly, a unique feature of the perilipin sequence that is not shared by other members of the protein family is the presence of multiple consensus sequences for the phosphorylation of serine residues by cAMP-dependent protein kinase [protein kinase A (PKA)]; the human (59), chimpanzee, and canine sequences of perilipin A include five sites, whereas the rat and mouse sequences include six sites (50, 58). The sequence of perilipin B includes only the first two or three PKA consensus sites (50, 58), depending on the species of animal; the second PKA site is not conserved in all species. Most published studies investigate the functions of perilipin A, the major isoform.

Sequence analysis shows that S3-12 is the most divergent member of the perilipin family of proteins (29). The S3-12 sequence with the highest similarity ($\leqslant$65%) to other members of the family includes a 33 amino acid sequence that is repeated at least 29 times in tandem in S3-12 (48), with shorter sequences in the other family members (Fig. 1). These sequences contain imperfect 11-mer repeats of amino acids that are predicted to form amphipathic helices with three helical turns per 11 amino acids (60). A similar sequence of seven 11-mer repeats in α-synuclein folds into amphipathic helices that mediate the association of α-synuclein with lipid vesicles and detergent micelles (60, 61); these sequences are also likely to mediate the binding of α-synuclein to lipid droplets (62). Based on biophysical studies of α-synuclein, the extensive tract of 11-mer repeats of S3-12 may be important for assembling S3-12 onto lipid droplets, with the hydrophobic faces of the helices embedded into the acyl chains of the phospholipid monolayer and the polar faces positioned at the aqueous interface of the lipid droplet with cytosol. However, targeting sequences have not yet been identified for S3-12, and the conformation of the protein at the lipid droplet surface is unknown. It is interesting, however, that deletion of part or all of the 11-mer repeat sequences reduced the targeting of nascent OXPAT/MLDP (46) but not TIP47 (40) or perilipin (54) to lipid droplets. Similar experiments provide ambiguous results for adipophilin; mutation of putative 11-mer repeat sequences reduced adipophilin targeting to lipid droplets in some studies (41, 42) but not in others (43). Regardless of whether or not the 11-mer repeat sequences are part of the molecular zip code that directs nascent proteins to a lipid droplet address, these sequences may play an important role in the positioning of these proteins at the surfaces of lipid droplets to optimally serve their functions.

FORMATION OF LIPID DROPLETS

Lipid droplets are thought to form within membranes of the endoplasmic reticulum. The final steps of neutral lipid synthesis are catalyzed by enzymes that reside in the endoplasmic reticulum. In mammals, two genes encode acyl-CoA cholesterol acyltransferases that catalyze the final step of cholesteryl ester synthesis (reviewed in Refs. 63, 64) and at least two genes encode acyl-CoA diacylglycerol acyltransferases that catalyze the final step of triacylglycerol synthesis (reviewed in Ref. 64). Although substantial evidence supports the synthesis of neutral lipids in the endoplasmic reticulum, less evidence supports the formation of lipid droplets within this compartment. The prevailing hypothetical model for lipid droplet formation proposes that neutral lipids accumulate in a lens-like pool between the lumenal and cytoplasmic leaflets of the phospholipid bilayer. The majority of evidence to support this model is derived from experiments using freeze-fracture electron microscopy to show that membranous structures consistent with endoplasmic reticulum membranes closely appose structures that can be identified as lipid droplets (24, 65, 66).

Several studies of lipid droplet morphology have concluded that lipid droplets remain in contact with the endoplasmic reticulum; however, in numerous studies, it is apparent that lipid droplets are found in close proximity not only to membranes of the endoplasmic reticulum but also to mitochondria and peroxisomes (24, 67). Furthermore, lipid droplets in mammalian cells, as well as *Drosophila* embryos, exhibit motility using dynein motors to move along microtubules (68–70). Additionally, lipid droplets of adipocytes show dramatic remodeling in response to the stimulation of adipocytes with β-adrenergic agonists (5, 71–73); a few large centrally located lipid droplets fragment into myriad tiny microlipid droplets that scatter throughout the cytoplasm (**Fig. 2**). Subsequent addition of the β-adrenergic antagonist propanolol reverses the process, promoting the migration of microlipid droplets back to residual large lipid droplets and eventual loss of the tiny droplets, presumably by fusion back into a few large droplets (D. L. Brasaemle, unpublished observation). The observed movement and dynamic remodeling of lipid droplets suggest that although lipid droplets may nucleate within the endoplasmic reticulum, they likely pinch off from endoplasmic reticulum membranes to become distinct structures. Transient contact of mature lipid droplets with other organelles has been suggested by recent studies showing

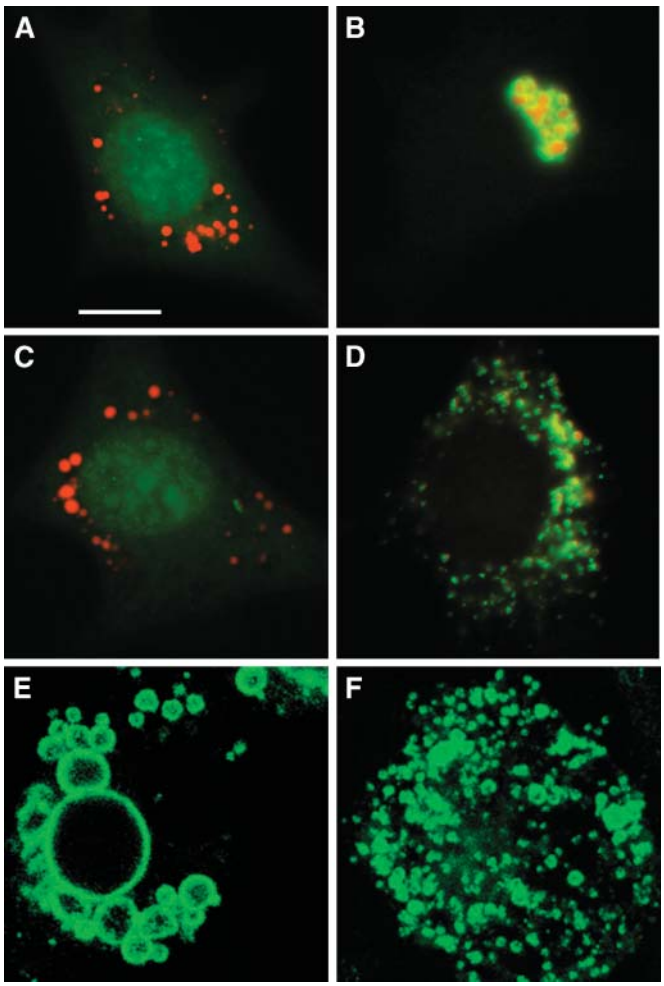

Fig. 2. Lipid droplet remodeling. Under basal conditions, lipid droplets form tight clusters when perilipin A (green) is ectopically expressed in fibroblasts (B); these lipid droplets fragment and disperse throughout the cytoplasm (D) after the activation of PKA. In contrast, lipid droplets in cells lacking perilipin (red) show a dispersed arrangement under both basal (A) and PKA-activated (C) conditions. Large perilipin-coated lipid droplets (green) of 3T3-L1 adipocytes under basal conditions (E) fragment and disperse throughout the cytoplasm (F) after the activation of PKA. Perilipin staining is shown in green; neutral lipid staining is shown in red. This figure is reproduced with permission from Marcinkiewicz et al. (72).

lipid droplet contact with peroxisomes in yeast (74) and may provide a mechanism to move lipids and proteins between lipid droplets and other compartments.

Lipid droplet maturation has been characterized in cultured 3T3-L1 adipocytes incubated with oleic acid, glucose, and insulin (32, 49). Interestingly, the composition of lipid droplet coat proteins changes as lipid droplets enlarge and mature. The earliest detectable deposits of neutral lipid collect in tiny dispersed structures near the periphery of adipocytes; both S3-12 and TIP47 colocalize to these minute structures (32, 49). As the lipid droplets grow, they migrate toward the center of the adipocytes and acquire adipophilin while gradually losing TIP47 content (32). As the droplets enlarge further, perilipin eventually replaces the other lipid droplet coat proteins; the largest centrally located lipid droplets are coated only with perilipin and not other members of the protein family (32). It is unclear whether lipid droplets increase in size after the acquisition of individual molecules or small parcels of neutral lipids or whether lipid droplet fusion occurs. However, as cultured adipocytes differentiate, many small lipid droplets are eventually "replaced" by a few large droplets, suggesting that the fusion of droplets is likely. The sequential progression of coat proteins during droplet formation and maturation suggests that each of these proteins may play different roles in facilitating lipid droplet formation and metabolism.

CYTOSOLIC LIPASES HYDROLYZE NEUTRAL LIPIDS STORED IN LIPID DROPLETS

When fatty acids or cholesterol are needed by cells or tissues, cytosolic lipases hydrolyze triacylglycerols and cholesteryl esters stored in lipid droplets. The most extensively characterized cytosolic lipase, hormone-sensitive lipase, is highly expressed in adipocytes and at lower levels in other cells and tissues (75, 76). Hormone-sensitive lipase has hydrolytic activity against a variety of substrates, including triacylglycerol, diacylglycerol, cholesteryl esters, and retinol esters (77–80). Early studies revealed that incubation of adipocytes with epinephrine or norepinephrine stimulated a massive increase in lipolysis, resulting in the release of fatty acids and glycerol from the cells (81, 82). During the past 50 years, numerous investigators have contributed to the elucidation of the pathway of β-adrenergic receptor-mediated signaling through G proteins to stimulate adenylyl cyclase, consequently activating PKA and increasing lipolysis in adipocytes (reviewed in Refs. 83–86). Hormone-sensitive lipase was identified as a substrate for PKA; phosphorylation of the protein modestly activates lipase activity (78, 87–92). However, the observed 2-fold increase of in vitro lipase activity after the phosphorylation of hormone-sensitive lipase does not explain the 50- to 100-fold increase in lipolysis after activation of the β-adrenergic receptor-mediated signaling pathway in intact adipocytes (86). Further investigation has revealed that phosphorylation of hormone-sensitive lipase on three serine residues (93) triggers the translocation of hormone-sensitive lipase from the cytosol to lipid droplets (94–96), where it gains access to its lipid substrates. Mutation of serine residues within the PKA consensus sites of hormone-sensitive lipase to alanines prevents both the translocation and docking of the lipase on lipid droplets and maximal lipolysis (97). Thus, the major function of this PKA-mediated phosphorylation is not activation of the enzyme but, instead, promotion of the translocation and docking of the lipase on lipid droplets.

For nearly three decades, hormone-sensitive lipase was thought to be the major adipocyte lipase catalyzing the hydrolysis of triacylglycerols to release fatty acids at times of energy deficit. In the early 2000s, study of hormone-sensitive lipase null mice by three different groups (98–100)

indicated that additional lipases contribute to lipolysis in adipocytes and other cells and tissues. Hormone-sensitive lipase null mice showed a complete absence of neutral cholesterol esterase activity in adipose tissue and testes and reduced, but not absent, triacylglycerol lipase activity in adipose tissue (98–100). Significantly, diacylglycerols accumulated in adipose tissue (98), indicating that hormone-sensitive lipase plays a critical role in diacylglycerol hydrolysis, leading to complete hydrolysis of triacylglycerols to fatty acids and glycerol. These observations illuminated the path to search for additional cytosolic triacylglycerol lipases.

In 2004, three groups simultaneously reported the identification of a new cytosolic triacylglycerol lipase (101–103) that has been named adipose triglyceride lipase (ATGL; also desnutrin). ATGL belongs to a larger gene family containing five members in vertebrates (101, 104–106), with similarity to lipases in insects (104) and also to the patatin family of acyl hydrolases in plants. ATGL is highly expressed in adipose tissue of mice and humans; it is expressed to a slightly lesser extent in heart, muscle, testis, adrenal gland, and colon; and it has very low but detectable expression in most other tissues (102, 103, 105, 107). Studies using intact cultured cells overexpressing ATGL, mammalian cell extracts containing ectopic ATGL, or purified recombinant ATGL have demonstrated that ATGL has triacylglycerol lipase activity (101–103, 105–107).

Studies using adipose tissue dissected from ATGL null mice (108) have supported a role for ATGL in lipolysis in both the presence and absence (basal conditions) of β-adrenergic receptor agonists. In contrast, studies using a specific inhibitor of hormone-sensitive lipase added to human adipocytes suggest that ATGL plays a greater role in basal or unstimulated lipolysis, whereas hormone-sensitive lipase is necessary for maximal catecholamine-stimulated lipolysis (109–111). Interestingly, excessive accumulation of triacylglycerols in the hearts of ATGL null mice leads to premature heart failure (108), supporting an essential role for ATGL in maintaining triacylglycerol homeostasis in the heart; thus, ATGL may play its most critical roles in cells other than adipocytes. In vitro studies of recombinant proteins (101) or overexpressed proteins immunoprecipitated from cultured cells (105) have shown that the ATGL family members adiponutrin, human GS2, and mouse GS2-like all exhibit triacylglycerol lipase activity. Additionally, ATGL and human GS2 have been demonstrated to have retinol ester hydrolase activity (112, 113). Because ATGL, adiponutrin, GS2, and GS2-like are all expressed in adipose tissue (105), the contributions of various lipases to adipocyte lipolysis are likely to be considerably more complex than once thought.

A CHANGING PARADIGM: LIPID DROPLET PROTEINS PARTICIPATE IN THE CONTROL OF LIPOLYSIS

Functional studies in cell culture and animal models have demonstrated that perilipin and the related proteins in the perilipin family regulate the lipolysis of stored neutral lipids. Ectopic expression of perilipin A in cultured fibroblasts that normally lack perilipin increases the triacylglycerol content of cells by slowing the rate of triacylglycerol turnover (114). One interpretation of these data is that perilipin forms a protective barrier on lipid droplets that restricts the access of cytosolic lipases to stored triacylglycerol. Mutagenesis studies have suggested that both N- and C-terminal sequences of perilipin A participate in providing this barrier function, because deletion of either region restores a more rapid rate of triacylglycerol degradation (55, 115). Adipophilin is the major surface protein on the lipid droplets of fibroblasts, but ectopic expression of perilipin A replaces the adipophilin content of the droplets in favor of a perilipin coat (116, 117). These data suggest that perilipin A competes with adipophilin for binding to lipid droplets and is more effective at attenuating lipolysis than adipophilin. Nonetheless, similar studies have shown that overexpression of adipophilin in a variety of cultured cells also increases triacylglycerol storage (118–122). These studies imply that adipophilin, like perilipin A, shields stored triacylglycerol from the activity of cytosolic lipases and, furthermore, that either adipophilin is expressed at limiting concentrations in cells or the surface concentration of adipophilin on lipid droplets can be increased through the expression of ectopic protein to afford a higher level of protection against lipolysis. Additionally, a recent study has shown that OXPAT/MLDP expressed ectopically in Chinese hamster ovary fibroblasts acts similarly to ectopic perilipin A in reducing triacylglycerol turnover (47). Presumably, OXPAT/MLDP also replaces adipophilin on the lipid droplet surface to afford a higher level of protection against cytosolic lipases.

Tumor necrosis factor-α (TNF-α) is a cytokine that contributes to the pathophysiology of both cancer cachexia and obesity. The mechanisms by which TNF-α functions are complex but include the alteration of lipolysis in adipocytes (123). Studies in cultured 3T3-L1 adipocytes have shown that TNF-α increases lipolysis in part by promoting the rapid degradation of perilipin mRNA and protein (124–126). When the perilipin coat cannot be repaired through the synthesis of new perilipin from endogenous mRNA, the barrier to lipolysis is disrupted. This barrier function can be restored by constitutive expression of ectopic perilipin A (125). Thus, loss of adipocyte perilipin content, with the concomitant loss of the protection of stored triacylglycerol from cytosolic lipases, is part of the mechanism by which TNF-α increases lipolysis; the resulting increased flux of fatty acids may contribute to local effects on gene expression within adipocytes as well as to distal effects of fatty acids on insulin sensitivity in other tissues.

Studies of perilipin null mice provide support for perilipin function in reducing basal lipolysis but also suggest that perilipin facilitates hormonally stimulated lipolysis. Perilipin null mice have ~30% of the fat mass of wild-type mice and significantly smaller average fat cell size (51, 52). Adipocytes isolated from perilipin null mice show increased rates of basal lipolysis (51, 52), consistent with a role for perilipin in providing a barrier function to reduce the access of cytosolic lipases to triacylglycerols stored in

lipid droplets. Interestingly, agonists to β-adrenergic receptors fail to maximally stimulate the release of glycerol and free fatty acids from perilipin null adipocytes (52), suggesting that perilipin also plays an important role in the mechanisms controlling hormonally stimulated lipolysis (**Fig. 3**).

In adipocytes, catecholamine-stimulated lipolysis is facilitated by the phosphorylation of several proteins by PKA. Phosphorylation of hormone-sensitive lipase by PKA facilitates the translocation of the lipase from the cytosol to dock on adipocyte lipid droplets, as discussed above. Adipocytes differentiated from perilipin null mouse embryonic fibroblasts (MEFs) have adipophilin-coated lipid droplets that fail to support the docking of hormone-sensitive lipase after the stimulation of β-adrenergic receptors (127). Additionally, activation of PKA facilitates fluorescence resonance energy transfer between fluorescent probes fused to hormone-sensitive lipase and perilipin A in live cultured cells (128). Together, these observations suggest that phosphorylated perilipin provides the docking site for phosphorylated hormone-sensitive lipase on lipid droplets (Fig. 3). By contrast, ATGL appears to be distributed between the cytoplasm and lipid droplets in adipocytes (103, 128) and other cells (105), whereas activation of PKA has little, if any, effect on the lipid droplet association of this lipase.

Perilipin A is both the most abundant protein on the surfaces of lipid droplets and the major PKA substrate in adipocytes (4, 129), suggesting that phosphorylated perilipin A serves important functions in catecholamine-stimulated lipolysis. Perilipin A has multiple PKA consensus sequences; however, it is currently unclear whether all of these sites are phosphorylated after the activation of PKA. Whereas rodent perilipin A has six PKA sites, the second PKA site is not conserved in primates or canines, suggesting that it may not serve a critical function. Mutagenesis studies provide circumstantial evidence for the phosphorylation of serines in at least three of the six PKA consensus sites in mouse

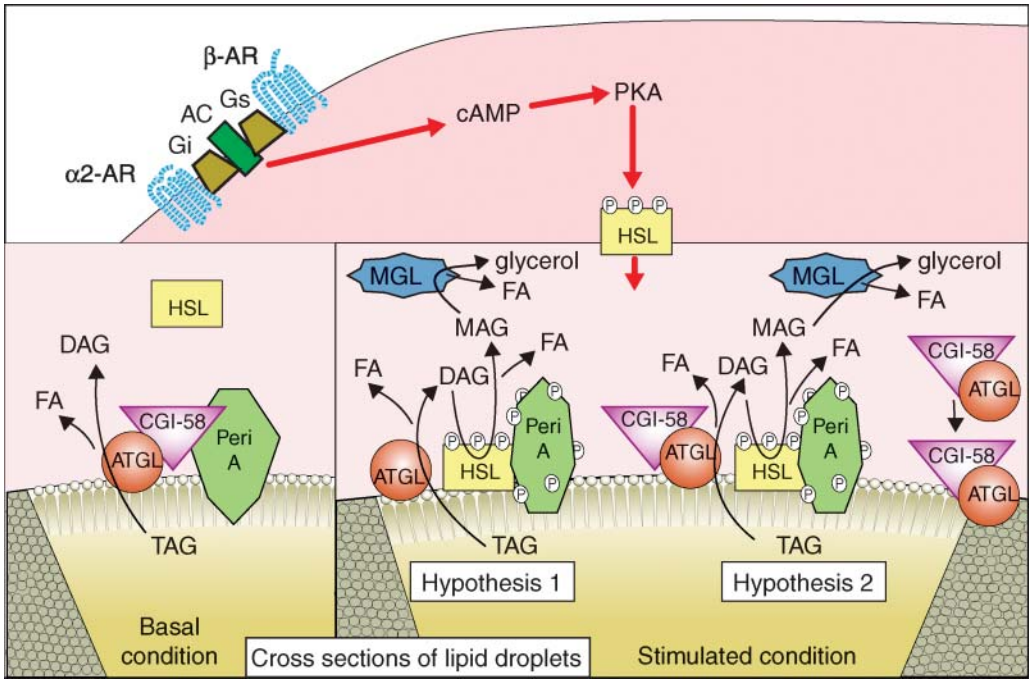

Fig. 3. Hypothetical model of basal and stimulated lipolysis in adipocytes. Under basal conditions (lower left panel), adipose triglyceride lipase (ATGL) associates with lipid droplets in a perilipin-independent manner. CGI-58 binds to perilipin A (Peri A) and facilitates basal lipolysis of triacylglycerol (TAG) by ATGL to produce diacylglycerol (DAG) and fatty acids (FA). Hormone-sensitive lipase (HSL) is cytosolic and has limited access to TAG stored within lipid droplets or DAG produced during basal lipolysis. When catecholamines bind to β-adrenergic receptors (β-AR), G protein (Gs)-mediated signaling activates adenylyl cyclase (AC) (upper panel). Increased cAMP levels activate cAMP-dependent protein kinase (PKA), which then phosphorylates HSL and perilipin A. Phosphorylated perilipin A changes conformation to facilitate increased lipolysis (lower right panel). Phosphorylated HSL docks on phosphorylated perilipin A and gains access to TAG and DAG substrates in lipid droplets. CGI-58 disperses off of lipid droplets into the cytoplasm. Two hypotheses are depicted for the roles of CGI-58 and ATGL in stimulated lipolysis. Hypothesis 1: CGI-58 disperses into the cytoplasm, where it has no further role in stimulated lipolysis. ATGL hydrolyzes TAG to DAG and FA. Further hydrolysis of DAG to monoacylglycerol (MAG) is catalyzed by HSL bound to perilipin A. Hypothesis 2: CGI-58 enters the cytoplasm, where it forms a complex with ATGL that binds to lipid droplets in a perilipin-independent manner. The ATGL/CGI-58 complex hydrolyzes TAG to DAG and FA. HSL hydrolyzes DAG to MAG and FA. For both hypotheses, MAG is hydrolyzed to glycerol and FA by monoglyceride lipase (MGL). Fatty acids and glycerol exit the adipocyte and enter the circulation. (Illustration by Rick Hasney.)

perilipin A, including one site at the N terminus and two sites at the C terminus. Studies in cultured fibroblasts ectopically expressing mutated forms of perilipin A have shown that simultaneous mutation of the three N-terminal PKA site serines to prevent phosphorylation reduces maximal lipolysis catalyzed by either hormone-sensitive lipase (55, 116) or the endogenous lipase of Chinese hamster ovary fibroblasts (117), presumably ATGL. Whereas one study shows that hormone-sensitive lipase fails to dock on lipid droplets coated with mutated perilipin that lacks the three N-terminal PKA sites (127), another study demonstrates hormone-sensitive lipase docking on lipid droplets coated with perilipin lacking all six PKA sites (130). However, in the latter study, lipolysis was not increased above basal levels after the activation of PKA, suggesting that the mere docking of hormone-sensitive lipase on lipid droplets is insufficient for maximal stimulated lipolysis. Together, these studies suggest that phosphorylation of perilipin A on one or more of the three N-terminal PKA site serines is critical for lipolysis catalyzed by hormone-sensitive lipase. Thus, perilipin A serves as a docking protein for hormone-sensitive lipase, but perilipin phosphorylation also facilitates lipase access to triacylglycerol substrates by an additional as yet uncharacterized mechanism.

Mutagenesis studies have shown that phosphorylation of at least two of the three C-terminal PKA sites of perilipin A contributes to the regulation of catecholamine-stimulated lipolysis. Mutation of all three of these PKA site serines to alanines (sites 4, 5, and 6) reduces PKA-activated lipolysis in cultured cells that lack hormone-sensitive lipase (55) but likely express ATGL and in adipocytes differentiated from perilipin null MEFs (131), which express both lipases. After chronic administration of β-adrenergic agonists to cultured 3T3-L1 adipocytes, PKA-mediated phosphorylation of the fifth PKA site serine triggers a massive remodeling of lipid droplets (Fig. 2); large perinuclear lipid droplets fragment into myriad perilipin A-coated microlipid droplets that disperse throughout the cytoplasm (72). Similar fragmentation of large lipid droplets has been observed in adipocytes in tissue dissected from rats after systemic infusion of β_3-adrenergic receptor agonists or after electroporation of β_1-adrenergic receptors into the fat pads of live rats (71).

Although a few studies have reported that perilipin sloughs off adipocyte lipid droplets into the cytoplasm after the stimulation of β-adrenergic receptors (95, 125), higher resolution microscopy and subcellular fractionation experiments have demonstrated that perilipin that exits the large central lipid droplets surrounds a small parcel of triacylglycerol (72). Association of perilipin with triacylglycerol stabilizes the protein (132), protecting it from degradation (133). Remodeling of lipid droplets may facilitate lipolysis by massively increasing the surface area of the microlipid droplets; because the mass of perilipin does not increase significantly, the barrier function of the perilipin coat is attenuated. Consistent with this hypothesis, ectopic expression of mutated perilipin lacking the fifth PKA site fails to support maximal stimulated lipolysis in adipocytes differentiated from perilipin null MEFs (131). Finally, mutation of the sixth PKA site serine, which is the final amino acid in the C terminus of perilipin A, reduces stimulated lipolysis to basal levels in adipocytes differentiated from perilipin null MEFs expressing endogenous hormone-sensitive lipase and ATGL, and also when the expression of either lipase is reduced by the use of small hairpin RNAs (131). Clearly, the phosphorylation of hormone-sensitive lipase is not the only control point for adipocyte lipolysis; perilipin A plays a critical role in controlling the access of lipases to lipid droplets.

PERILIPIN A AS A DYNAMIC SCAFFOLD SURROUNDING ADIPOCYTE LIPID DROPLETS

During the past 18 years, research into the biochemistry of lipid droplets has demonstrated that perilipin serves a critical role in regulating basal and stimulated lipolysis in adipocytes. The mechanisms by which perilipin attenuates lipolysis under basal conditions, facilitates hormone-sensitive lipase docking and activity, and triggers the remodeling of lipid droplets are poorly understood. One hypothesis proposes that perilipin forms a dynamic scaffold surrounding lipid droplets that serves as an organizing center to control the access of enzymes to lipid droplets (134). Much like a classic adaptor protein's role in facilitating cargo sorting into vesicular compartments, perilipin may coordinate the recruitment of proteins to the lipid droplet in a manner that is tuned to the metabolic status of the adipocyte. Under basal conditions, perilipin may bind proteins that facilitate triacylglycerol storage while allowing a low level of lipolysis. When PKA is activated, phosphorylated perilipin disperses the basal coat proteins to make way for powerful lipolytic machinery, including lipases and trafficking molecules, some of which may facilitate the budding off of microlipid droplets.

Two examples of perilipin binding proteins have been reported. Hormone-sensitive lipase requires perilipin A to dock on lipid droplets and gain access to lipid substrates after hormonal stimulation of adipocytes, as discussed. An example of a protein that binds to perilipin A under basal conditions is CGI-58, also called ABHD5 (for α/β hydrolase fold domain 5) (134, 135). CGI-58 plays a poorly understood role in the catabolism of triacylglycerol. Mutations in CGI-58 are responsible for a rare human disorder called Chanarin-Dorfman syndrome (or neutral lipid storage disorder) that is characterized by ichthyosis and excessive accumulation of triacylglycerols in many cells and tissues (136), although not adipose tissue. Thus, CGI-58 plays an important role in triacylglycerol homeostasis in many cells and tissues. Early studies of fibroblasts from humans with Chanarin-Dorfman syndrome demonstrated that turnover of triacylglycerols is impaired (137–139), primarily as a result of increased recycling of fatty acids and diacylglycerol released during lipolysis back into triacylglycerol biosynthesis (138, 139). More recent studies suggest that CGI-58 serves as a coactivator of ATGL, stimulating triacylglycerol hydrolysis by as much as 20-fold (140, 141).

Interestingly, although CGI-58 binds to perilipin A on adipocyte lipid droplets under basal conditions, it rapidly

disperses into the cytoplasm after stimulation of β-adrenergic receptors (73, 134). Since ATGL is constitutively associated with lipid droplets, this unexpected translocation implies that either CGI-58 activates ATGL under basal (Fig. 3) but not lipolytically stimulated conditions or CGI-58 plays a more complex role in triacylglycerol catabolism that may not be linked directly to ATGL activity. An alternative hypothesis (128, 141) is that perilipin may sequester CGI-58 at the lipid droplet under basal conditions, preventing its interaction with ATGL; under lipolytically stimulated conditions, the release of CGI-58 from the lipid droplet surface facilitates its interaction with ATGL in the cytosol, creating an active enzyme complex that can then bind to lipid droplets in a perilipin-independent manner (Fig. 3). Differences between the phenotypic traits of humans with CGI-58 mutations (136) and ATGL mutations, most notably a lack of ichthyosis in the latter (142), suggest that CGI-58 may function independently from ATGL. Furthermore, the expression of CGI-58 in numerous tissues (134) that lack perilipin implies that perilipin may coordinate CGI-58's association with lipid droplets in adipocytes but is not required for its activity.

How the phosphorylation of perilipin A alters the recruitment and binding of proteins and leads to the remodeling of lipid droplets has not yet been elucidated. Clues to some of these mechanisms may be gathered from recent studies of related proteins in insects. Surprisingly, lipid droplet structural proteins, lipases, and the above-described mechanisms controlling lipolysis are remarkably conserved in insects. Insects express two proteins related to perilipins, LSD1 and LSD2 (143–145); these proteins coat the lipid droplets in embryos and the fat bodies (tissues that resembles adipose tissue) of larval and adult insects. Deletion of LSD2 from *Drosophila* yields lean flies (143, 145), whereas overexpression of LSD2 produces obese flies with up to 50% more triacylglycerol in fat body stores (143). Lipolysis is acutely regulated by adipokinetic hormone (144, 146), a G protein-coupled receptor that activates adenylyl cyclase and, consequently, PKA. In *Manduca sexta*, LSD1 is phosphorylated by PKA, leading to the activation of lipolysis (144); the lipase involved in this stimulated lipolysis has not yet been identified. Insects have a triacylglycerol lipase similar to ATGL, encoded by the *brummer* gene, that is highly expressed during starvation and that, when deleted, leads to obesity (104). This lipase appears to have constitutive activity that is unaffected by PKA. Thus, the general mechanisms that control both basal and stimulated lipolysis are conserved between insects and vertebrates.

Clues to factors influencing lipid droplet motility may also be gained from the study of insects. *Drosophila* LSD2 is phosphorylated by a kinase called Halo; phosphorylated LSD2 plays a role in facilitating lipid droplet motility along microtubule tracks during the development of *Drosophila* embryos (147). This function is attributable in part to a binding interaction of LSD2 with the Klarsicht protein, which coordinates the activity of motor proteins associated with lipid droplets (147, 148). By extrapolation, it is tempting to speculate that phosphorylation of the serine residue in the fifth PKA site of perilipin A may trigger lipid droplet remodeling by recruiting a complex of proteins, including motor proteins, that pull apart large lipid droplets to disperse microlipid droplets along cytoskeletal tracks.

Whether the scaffold hypothesis applies to other members of the perilipin protein family remains to be determined. The sequential lipid droplet association of at least four perilipin family members during lipid droplet formation in adipocytes (32, 49) suggests that these proteins may serve distinct functions during lipid droplet maturation, perhaps by recruiting different subsets of adipocyte proteins that assist lipid droplet growth and inward migration to form large central perilipin-coated droplets. The stability of the exchangeable lipid droplet proteins, including TIP47, S3-12, and OXPAT/MLDP (36), in the cytoplasm likely facilitates rapid protein association with the earliest deposits of neutral lipids and may be essential for efficient lipid packaging and the stabilization of nascent droplets. By contrast, the integral lipid droplet proteins, perilipin and adipophilin, may serve more critical roles in managing the turnover of neutral lipid stores to facilitate the regulated release of fatty acids and cholesterol in response to changes in the metabolic state. Members of the protein family show distinct tissue expression patterns, implying that the proteins have most likely evolved to serve different functions in these tissues; whereas adipophilin and TIP47 are expressed ubiquitously, OXPAT/MLDP, perilipin, and S3-12 are expressed in only a few tissues that make use of specific signaling pathways to regulate lipogenesis and lipolysis. Finally, the conservation of this protein family throughout the evolution of both invertebrates and vertebrates highlights the importance of the efficient management of fat stores to survival.

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