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Minireview

A proposed model of fat packaging by exchangeable lipid droplet proteins

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Abstract Humans have evolved mechanisms of efficient fat storage to survive famine, but these mechanisms contribute to obesity in our current environment of plentiful food and reduced activity. Little is known about how animals package fat within cells. Five related structural proteins serve roles in packaging fat into lipid droplets. The proteins TIP47, S3–12, and OXPAT/MLDP/PAT-1 move from the cytosol to coat nascent lipid droplets during rapid fat storage. In contrast, perilipin and adipophilin constitutively associate with lipid droplets and play roles in sustained fat storage and regulation of lipolysis. Different tissues express different complements of these lipid droplet proteins. Thus, the tissue-specific complement of these proteins determines how tissues manage lipid stores.

Keywords: Lipid droplet; PAT proteins; Triacylglycerol; S3–12; TIP47; Perilipin; ADRP; Adipophilin; OXPAT; MLDP; PAT-1

1. Introduction

Human behavior and nutrient storage mechanisms are adapted to survive famine. When food is available, people consume quantities in excess of their immediate energy needs and store the excess energy as triacylglycerol (TAG) within the lipid droplets of adipose tissue. These behaviors and the metabolic mechanisms required to survive food scarcity have resulted in widespread obesity in an environment that features plentiful food and low demands for energy utilization. An emerging concept is that in obesity the TAG storage capacity of adipose depots, especially the visceral depot, overflows, thereby releasing free fatty acids and resulting in accumulation of TAG in non-adipose tissues (steatosis) [1–4]. Non-adipocytes are poorly adapted to store excess TAG and may sustain lipotoxic disruption of cellular function by mechanisms that are being actively investigated [5]. Lipotoxicity has been implicated in the pathogenesis of the features of the metabolic syndrome, including dyslipidemia, insulin resistance, β-cell failure, and hypertension. We hypothesize that the capacity of the intracellular TAG droplet compartment is finite in adipocytes, so that excess free fatty acids circulate to and are taken up by peripheral tissues, where they contribute to the metabolic sequelae of obesity.

This review focuses on TAG packaging rather than other neutral lipids, since TAG packaging or fat storage, plays a central role in whole body energy homeostasis. We explore the hypothesis that a set of proteins moves from the cytosol to the TAG/aqueous interface of nascent lipid droplets during rapid TAG synthesis, and that these proteins are necessary to effectively sequester newly synthesized TAG and to facilitate delivery of TAG to mature lipid droplets. Mature lipid droplets are coated by a separate set of proteins that delimit the TAG/aqueous interface. We, and others, hypothesize that the proteins that coat mature lipid droplets control access of metabolic enzymes to the stored TAG that they enclose. Finally, we include speculation on the roles of these lipid-binding proteins in regulation of energy metabolism.

2. Why triacylglycerol?

Animals store the bulk of their energy as fatty acids esterified to glycerol, or TAG. TAG is an efficient energy storage molecule, because its hydrophobicity allows tight packing into droplets without rigidity. Consequently, the energy to mass ratio of TAG is at least 10 times that of hydrated carbohydrates or proteins. Lipid droplets have a structure similar to that of lipoproteins. The TAG-rich core is surrounded by a single layer of phospholipids with their hydrophobic acyl-chains dissolved in the TAG core and the hydrophilic head groups interfacing with the aqueous cytosol. Just as lipoproteins are embedded with apolipoproteins, intracellular lipid droplets are surrounded by specific proteins. Some of these proteins embed hydrophobic anchors into the hydrophobic acyl-chains of the phospholipid monolayer and the hydrophobic core of lipid droplets, whereas other proteins associate electrostatically with the head groups or with the hydrophobically anchored proteins. Examples of hydrophobically-anchored proteins include adipophilin (also known as adipocyte differentiation-related protein, ADRP) [6–8], perilipin [9], the caveolins [10,11], and the hepatitis C virus core protein [12,13] in animals, as well as the oleosins [14,15] in plants. On the other hand, proteins such as CGI-58, and likely hormone-sensitive

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lipase, associate with lipid droplets by forming electrostatic complexes with perilipin [16,17]. Evidence is mounting that the proteins embedded into lipid droplets help sequester esterified fatty acids [9,18–20].[1] Among these proteins, some drive TAG storage, whereas others regulate TAG hydrolysis. Thus, TAG packaging into droplets likely protects cells from detrimental effects of excess unesterified fatty acids, such as alterations in intracellular osmolarity, disruption of membranes, partitioning of excess substrate into prostaglandin and leukotriene synthetic pathways thus altering subsequent signaling, alteration of fatty acid signaling through peroxisome proliferator activated receptors (PPAR) and G-protein-linked fatty acid receptors, production of lipid metabolites that alter signaling pathways, and increased production of reactive oxygen species by fatty acid oxidation. Unesterified fatty acids are highly toxic to cells [21] and have been implicated in much of the pathology associated with obesity and diabetes, as noted above. Thus, efficient fatty acid esterification into TAG and packaging of the newly synthesized TAG are necessary for viability of cells and likely of whole organisms.

3. TAG is coated by lipid binding proteins

The hydrophobicity of TAG and other neutral lipids such as sterol esters necessitates elaborate mechanisms to emulsify and transport these molecules through the aqueous compartments of organisms. These mechanisms include the extensively studied lipoprotein system for extracellular transport of these lipids. TAG and cholesterol esters are emulsified and packaged during lipoprotein synthesis in the lumen of the secretory pathway [22]. Secreted lipoproteins then deliver TAG and other lipids to tissues. Extensive study of this process has contributed to a detailed understanding of how apolipoproteins bind to the emulsified lipids and associate with lipoproteins, how apolipoproteins mediate remodeling of circulating lipoproteins, how the association of lipoproteins with cell surface receptors mediates internalization, and how lipoproteins are metabolized after uptake.

Surprisingly, how TAG and other neutral lipids are packaged into cytosolic droplets is not well understood. However, the vast majority of TAG in mammalian cells is in droplets coated with one or more of the perilipin–adipophilin-TIP47 (PAT) family of proteins [23]. This protein family is defined by sequence similarity and lipid droplet binding. The observations that the adipocyte protein S3–12 coats nascent lipid droplets and shares sequence similarity with both TIP47 and adipophilin puts S3–12 [24–26] in the PAT family. Myocardial lipid droplet protein (MLDP) is yet another PAT protein that we[2] and other groups [23,27] have independently identified by sequence homology to the PAT family. MLDP is also termed PAT-1 in the NCBI database (Accession no. AY919875). We have referred to this protein as OXPAT in national meetings and in the text that follows, in accordance with its expression in highly oxidative tissues and during states of increased fatty acid oxidation[2]. OXPAT expressed in a heterologous cell system binds lipid droplets[2] [27]. Each of the PAT proteins has a unique tissue distribution, subcellular localization, and lipid binding properties, which suggests that each has a unique role in TAG management. Since energy is required for the survival of all organisms and food energy limits most animal populations, the management of energy reserves has been a powerful selective force. Thus, it is not surprising that mammalian genomes have at least five genes that encode regulatory proteins that coat TAG droplets.

4. PAT proteins may be constitutively bound to TAG droplets or bind to expanding lipid droplets during rapid TAG synthesis

The five PAT proteins can be divided into two categories by their stability when not bound to droplets. Perilipin and adipophilin are generally found only in cells with neutral lipid pools and only bound to lipid droplets. In the absence of neutral lipid these proteins are targeted to proteasomes for degradation [28–33]. Thus, perilipin and adipophilin will be referred to as constitutively TAG-associated PAT proteins (CPATs). The position of perilipin and adipophilin at the interface between the cytosol and the surface of lipid droplets is ideal to regulate this pool. Multiple lines of evidence show that perilipin exerts primary control over adipose TAG stores [9,19,20], whereas adipophilin is the major CPAT in most other tissues [29]. When perilipin, the major adipocyte CPAT, is ablated in mice, it is replaced on TAG droplets by adipophilin [20]. Mice lacking perilipin do not accumulate large adipose TAG stores and hormone-regulated lipolysis is muted [19,20,34]. These data and others show that perilipin is effective in protecting TAG from hydrolysis in adipocytes of fed animals and orchestrates hormone-stimulated lipolysis in fasted animals or following β-adrenergic stimulation. The mechanisms by which perilipin exerts control over TAG stores have been the subject of extensive research by numerous investigators; coverage of these studies is beyond the scope of this minireview and the reader is referred to several recent reviews [35,36]. Taken together, these data suggest that perilipin controls the primary TAG stores of animals from its position at the interface between the cytosol and the TAG droplets of adipocytes.

Adipophilin in non-adipocytes protects the TAG it encloses from hydrolysis by cellular lipases, analogous to the barrier role played by perilipin in adipocytes. Adipophilin is constitutively associated with TAG droplets in non-adipocytes [29,37]. Overexpression of adipophilin, like overexpression of perilipin, increases intracellular TAG stores in cultured cells [18,38,39]. Furthermore, adipophilin-null mice, which presumably have no CPATs in most tissues including the liver, are less effective at storing TAG in liver and likely other non-adipose tissues [40]. In comparison to perilipin, adipophilin is less protective against TAG hydrolysis; adipophilin-coated lipid droplets in the adipocytes of perilipin-null mice store less TAG due to rapid basal turnover [20]. Moreover, when perilipin is overexpressed in cultured cells, perilipin replaces adipophilin on TAG droplets and cellular TAG stores increase [9]. In contrast to perilipin, adipophilin does not play a role in hormone-stimulated lipolysis; perilipin-null mice with adipophilin-coated droplets in their adipocytes display a muted lipolytic response

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[20,34]. Thus, the CPATs control access of lipases to the TAG they delimit, but they are not interchangeable. Within a given tissue, the relative expression of the CPATs determines how much TAG a tissue is capable of storing.

Unlike the CPATs, TIP47, S3–12 and OXPAT are stable cytosolic proteins when the TAG pool is quiescent[2] [25–27,37]. Supplementing cultured cells with long-chain fatty acids results in rapid TAG synthesis and the emergence of new cytosolic droplets. These nascent droplets are coated with various combinations of TIP47 [26,37], S3–12 [25,26], and OXPAT,[2] depending upon which of these proteins are expressed in a given cell. TIP47, S3–12, and OXPAT move from a pre-existing cytosolic pool to form the protein coat of nascent TAG droplets. New protein synthesis is not required for the formation of nascent TAG droplets during lipid-loading of 3T3-L1 adipocytes [26]. Since TIP47, S3–12, and OXPAT exchange between the cytosol and the surface of TAG droplets, they will be referred to as the exchangeable TAG-associating PAT proteins (EPATs). This translocation requires long-chain fatty acids (16–20 carbons); medium-chain (10–14 carbons) and short-chain (8 or fewer carbons) fatty acids are poorly incorporated into TAG and minimally affect the cellular distribution of EPATs. It is interesting to note that TIP47 was originally reported as being required for movement of the mannose-6-phosphate receptor between endosomes and the trans-Golgi network [41]. Recent data support a role for TIP47 in regulating the localization of Rab9 [42], which in turn has been implicated in regulation of sterol trafficking [43]. Thus, the role of TIP47 in cellular lipid metabolism may be even more complex than its cycling between the cytosol and lipid droplets suggests.

Given the above data, we hypothesize that EPATs provide an empty reservoir that allows TAG storage to occur unfettered by the need to synthesize new droplet coat proteins. Increasing the size of the reservoir by TIP47 overexpression allows cells to accumulate TAG more rapidly.[1] Also, we have found that TAG storage driven by incubation with long chain fatty acids has a rapid initial phase and then dramatically slows.[2] As the reservoir for new TAG fills, there must be a mechanism by which the nascent droplets transfer the TAG within to the preexisting storage droplets. We have observed live 3T3-L1 adipocytes by microscopy during rapid TAG synthesis; over a period of 100 min, we observed no clear instances of fusion between nascent droplets or between small nascent droplets and larger storage droplets [26]. These data do not rule out droplet fusion as a mechanism for growth of TAG droplets or transfer of TAG between droplets. Other potential mechanisms of droplet growth include the synthesis of new TAG in the droplet or transfer of TAG from the site of synthesis via lipid transfer proteins. Regardless of the mechanism, it is likely that the EPATs ultimately mediate delivery of nascent TAG to the CPAT-delineated storage depot.

The need for EPATs in animals contrasts with lipid storage in the oil seeds of plants. Over the growing season, many plants convert solar energy into chemical energy and store it in TAG droplets, termed oil bodies, in seeds. Plant oil bodies are coated by the oleosins, which are proteins that constitutively embed deeply into lipid bodies via a central hydrophobic hairpin structure with a proline knot [44–47]; oleosins are thought to function to prevent oil body fusion during seed dessication. Animals must cope with unpredictable feeding opportunities, and so animal cells must be able to handle boluses of metabolic fuels, such as glucose and fatty acids. Plants, in contrast, have a more constant source of energy—the sun. Notably, no EPATs have been reported for oil seeds, likely because plants do not need to accommodate feeding derived boluses of TAG and the consequent rapid lipid packaging into droplets.

5. Proposed lipid binding mechanisms for PAT proteins

Consistent with the ability of EPATs to reversibly bind to lipid droplets, TIP47 has two predicted lipid binding structures characterized by amphipathic helices [48,49] that may mediate this reversible binding. One of these lipid binding structures resides in the amino-terminal half of the protein and is postulated to be an elongated helix formed by 11-mer repeats. Bussell and Eliezer identified these 11-mer repeats in multiple exchangeable lipid binding proteins, including apolipoproteins, TIP47, adipophilin, perilipin, S3–12, and α-synuclein [49]. They proposed that the 11-mer repeats form unusual right-handed helices with 3 turns per repeat, thereby generating a TAG miscible face and a water miscible face [49]. Notably, approximately two-thirds of the amino acid sequence of the 160 kDa S3–12 consists of these 11-mer repeats, which were initially described as a 33-mer repeating motif [24]. Experiments are needed to determine whether the 11-mer repeats in S3–12 and the other PAT proteins are necessary and/or sufficient for lipid droplet binding.

The other predicted lipid binding structure in TIP47 resides in the carboxyl-terminal half, and its crystal structure has revealed a 4-helix bundle [48]. This 4-helix bundle has significant structural similarity to an amphipathic 4-helix bundle present in the exchangeable apolipoprotein apoE. These amphipathic helices can either open, splay out and embed into the surface of the lipoproteins or fold to retract their hydrophobic surfaces, which renders these folded molecules stable in solution. This retractable lipid binding domain allows apoE to coat the lipoprotein surface in TAG-rich lipoproteins and to release from the particle as TAG is hydrolyzed and the lipoprotein particle shrinks [50]. Similarly, TIP47 is stable both bound to lipid droplets and when soluble in the cytosol, and TIP47 efficiently transfers between both compartments. TIP47 rapidly coats nascent TAG droplets, presumably when lipid binding sites are created as the nascent TAG droplet surface expands. Furthermore, S3–12 and OXPAT bear sequence similarity to TIP47 across these lipid-binding structures and, like TIP47, coat nascent TAG droplets. These parallels with apoE suggest conserved mechanisms and functions for apoE, TIP47 and S3–12 in their exchangeable binding of micelles of lipid and protein.

6. PAT proteins organize TAG storage

Immunostaining of PAT proteins in cultured adipocytes reveals spatially and temporally organized TAG packaging [25,26] (Fig. 1). Under basal conditions, the adipocyte lipid droplet architecture is homogeneous—all of the TAG is in large, centrally located perilipin coated lipid droplets and the EPATs are cytosolic. However, when cultured adipocytes are incubated with long-chain fatty acids, within 10 min, small TAG-filled puncta emerge that are both uniform in size

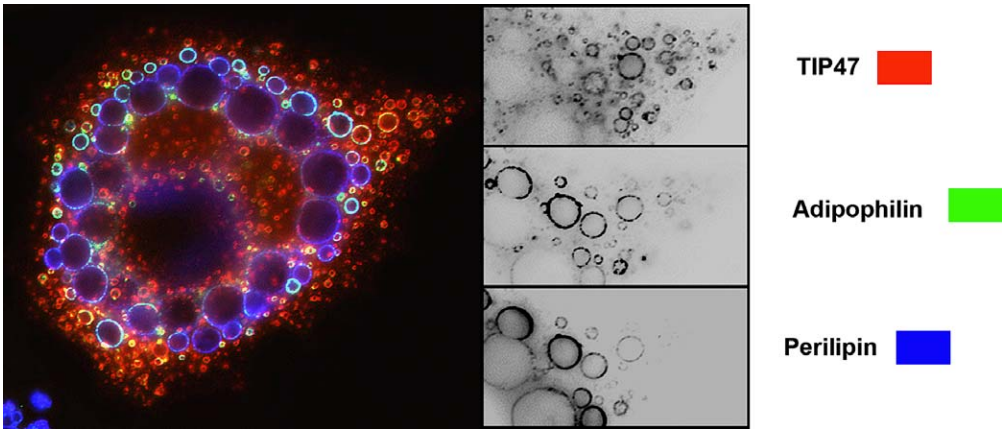

Fig. 1. PAT proteins reveal ordered TAG packaging. TAG synthesis was driven by adding albumin-bound oleate to the basal medium of 3T3-L1 adipocytes. After 2 h of oleate loading, the adipocytes were immunostained with antibodies raised against perilipin (shown as blue) adipophilin (shown as green) and TIP47 (shown as red). *Note*: *OXPAT and S3–12 are also expressed in adipocytes and thus, this image shows only part of the complexity of PAT-mediated TAG packaging.*

and have a uniform coat composed of TIP47, S3–12 and adipophilin. These puncta are concentrated at the base and in the periphery of adipocytes, and are distinct from the pre-existing perilipin-coated droplets. Over the next hour of long-chain fatty acid treatment, TIP47, S3–12, and adipophilin partially resolve from one another. TIP47 and S3–12 are concentrated on the smallest, most peripheral droplets; additionally S3–12 extends to some of the larger, more central droplets. Adipophilin is concentrated on droplets intermediate in size and location between the smaller, peripheral TIP47/S3–12 coated droplets and the large, central perilipin-coated droplets. At 1 h, the nascent droplets remain largely separate from the perilipin-coated droplets. However, over the next few hours, mixing of the nascent droplet pool and the perilipin droplet pool is evidenced by emergence of droplets coated with S3–12, adipophilin, and perilipin. Thus, after several hours, a gradient of TAG droplets is established with TIP47 and S3–12 concentrated on the smallest most peripheral droplets, adipophilin concentrated on a ring of intermediate sized droplets, and perilipin concentrated on the large, perinuclear droplets (Fig. 1). When long-chain fatty acid is removed from the adipocyte media, adipocytes return readily over 30–180 min to their homeostatic lipid droplet architecture, with S3–12 in the cytosol and all of the TAG packaged in perilipin-coated lipid droplets [25,26]. We assume that the absence of TIP47 and S3–12 from the large, central lipid droplets reflects their release from the droplet surface during droplet maturation, but definitive experiments to prove this point remain to be completed.

We hypothesize that PAT family proteins not only reveal, but actually order TAG packaging by managing the droplet interface with the cytosol. As noted above, perturbing levels of the PAT proteins alters how cells and whole organisms manage TAG. Cells that have a high flux of lipids through storage and/or metabolic pathways, such as adipocytes, hepatocytes, muscle cells, and steroidogenic cells, express several PAT proteins[2] [25,27,51], whereas cells not specialized for TAG management express only adipophilin and TIP47. PAT family proteins are encoded by five different genes and alternative splicing results in many additional PAT protein isoforms that are expressed in a tissue-specific manner. Some PAT proteins are modified post-translationally by phosphorylation, and may undergo other types of regulated processing, such as acylation. Additionally, PAT proteins have been shown to bind other proteins, thereby recruiting these proteins from the cytosol to the droplet [16,17,34]. For example, the perilipin A isoform binds CGI-58 on the surface of adipocyte lipid droplets, and CGI-58 is released from lipid droplets during lipolytic stimulation [17]. Recently, CGI-58 has been reported to activate a major triglyceride lipase, adipocyte triglyceride lipase [52]. Hence, a function of PAT proteins may be to regulate access of enzymes and their activators to the TAG stores; the cell-specific complement of PAT proteins likely determines what proteins are recruited to the lipid droplet surface, and thereby how cells manage intracellular lipid metabolism.

PPARs sense cellular free fatty acid levels and, when high levels are detected, PPARs drive transcription of genes involved in fatty acid metabolism [53]. Consistent with the notion that PAT proteins influence free fatty acid levels in cells, PPAR activation boosts transcription of all of the PAT genes except TIP47 [54–56]. If, as we hypothesize, EPATs drive TAG storage and CPATs control TAG release, then under non-lipolytic conditions PAT proteins would be predicted to lower cellular free fatty acid levels by sequestering esterified fatty acid, thereby reducing endogenous ligands for the PPARs. Alternatively, it is possible that specific PAT proteins might compartmentalize esterified fatty acids for delivery and ultimate release of ligands to PPARs. These possibilities are being explored experimentally. Regardless of the relationship between PAT proteins and PPARs, the influence of PAT proteins extends beyond the interface of the lipid droplet with the cytosol. In support of this point, genetic ablation of perilipin in mice, which results in a lean phenotype and resistance to genetic [19] and diet-induced [20] obesity, is associated with significant alterations in gene expression of oxidative and lipid biosynthetic pathways, not only in white adipose tissue, but also in peripheral tissues, such as liver, heart, skeletal muscle, and kidney [57]. These changes result in augmented β-oxidation of fatty acids in white adipose tissue, liver, and muscle [58]. Furthermore, perilipin null mice display increased leptin secretion from adipocytes [20].

7. Lipid droplets likely emerge from the ER

Historically, it has been proposed that lipid droplets emerge from the endoplasmic reticulum (ER) as a lens of TAG between the ER membrane leaflets that, at some point during expansion, buds as a droplet into the cytosol [59,60]. This model has been largely based on morphological observations that mature TAG droplets are surrounded by and in some cases appear to be continuous with the ER [26,61–63]. This model was also based on the observation that diacylglycerol acyltransferase (DGAT) and other neutral lipid synthetic activities fractionate with the ER [64–66]. More recently, many enzymes of neutral lipid biosynthetic pathways have been identified and cloned, and confirmed to reside in the ER [67,68]. These data are consistent with lipid droplet formation being initiated in the ER. Immunoreactive puncta for S3–12, TIP47, and adipophilin arise after a few minutes of long-chain fatty acid driven TAG synthesis in adipocytes [26]. These puncta grow into TAG droplets as adipocytes synthesize additional TAG. Our working hypothesis is that TAG-filled lipid droplets bud from the ER membrane at specific exit sites and that the puncta of PAT proteins observed within a few minutes of lipid-loading correspond to these exit sites. Furthermore, other investigators have shown that a dominant negative form of caveolin shows a reticular localization under lean culture conditions, but localizes to puncta of nascent lipid droplets along the ER soon after addition of fatty acids to cultured cells [10]. This topographical restriction is reminiscent of that described previously for COPII-coated vesicle export from the ER [69]. These observations support the historical model of TAG droplet emergence from the ER, but also highlight parallels between packaging of TAG-cored lipid droplets and of aqueous-cored cargo vesicles (Fig. 2).

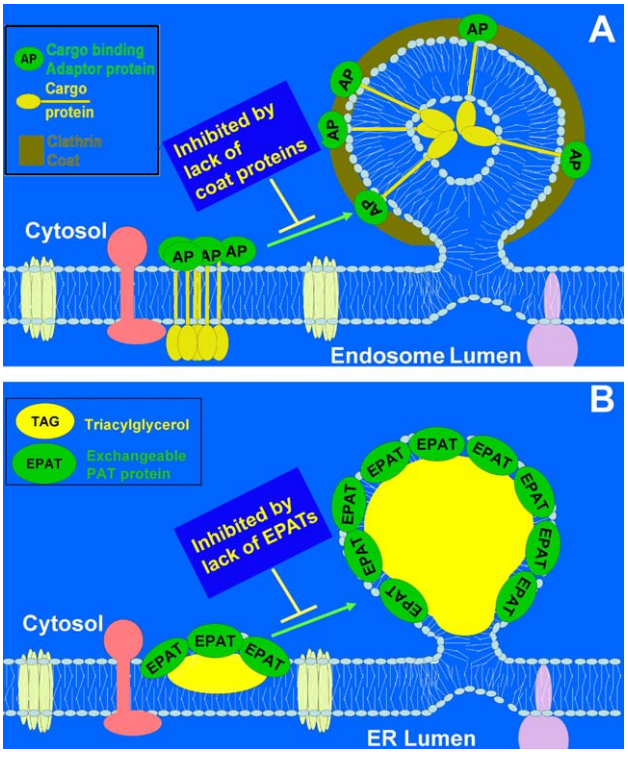

Fig. 2. Models of aqueous-cored vesicle and TAG-cored lipid droplet formation. (A) Depicts the formation of an aqueous-cored vesicle from an endosomal membrane. (1) Adaptor proteins bind cytosolic tails of transmembrane cargo proteins. (2) Adaptor proteins concentrate transmembrane proteins destined for the vesicle into a patch. (3) Adaptor proteins recruit the clathrin coat. (4) Clathrin coat mediates vesicle budding. (B) Depicts the formation of a TAG-cored lipid droplet. (1) EPATs concentrate TAG into a lens in the ER membrane. (2) EPATs, likely with the help of cytosolic proteins, mediate budding of TAG-cored lipid droplets.

8. Parallels between trafficking of TAG-cored lipid droplets and aqueous-cored vesicles

Viewed from the cytosol, TAG-cored lipid droplets and aqueous-cored vesicles are similar in that both structures present a curved protein-coated phospholipid layer to the cytosol. We suggest that TAG-cored lipid droplets and aqueous-cored vesicles arise by analogous mechanisms and may utilize some of the same cytosolic trafficking machinery. Formation of aqueous-cored vesicles that contain specific cargo occurs in stepwise fashion. First, cytosolic adaptor proteins bind to the cytosolic tails of transmembrane proteins, thereby concentrating these proteins into patches within the donor membranes (Fig. 2A). These transmembrane proteins pull along the appropriate luminal proteins via protein-protein interactions to form cargo-rich patches. Next, the adaptor proteins recruit the remainder of the cytosolic protein coat to these cargo-rich patches and the vesicle buds from the donor membrane. Proteins on the surface of the vesicle recruit the cytosolic machinery that directs the vesicle to the appropriate acceptor membrane.

Delivering TAG from the ER to constitutive TAG storage droplets likely requires many of the same steps as aqueous-cored vesicle trafficking (Fig. 2B). In our working model, we speculate that EPATs bind to TAG-rich ER membranes and concentrate this hydrophobic cargo into patches, just as adaptor proteins concentrate aqueous cargo into patches on the donor membrane. Next, by means of electrostatic interactions, EPATs may recruit the cytosolic molecular machinery that buds these TAG-filled droplets from the ER membrane and directs them to the CPAT compartment, just as adaptor proteins recruit the proteins that bud aqueous-cored vesicles from donor membranes and direct them toward acceptor membranes (Fig. 2B).

This hypothesis is driven by a growing number of observations in the literature that suggest that vesicular trafficking and lipid droplet biogenesis may utilize many of the same proteins. For example, proteins implicated in trafficking of aqueous-cored vesicles also have been found in TAG droplets, including numerous small G-proteins, EHD2, α-SNAP and Sec22 [70,71]. Although demonstration of physiologic association of these proteins with lipid droplets is required to confirm the model, it is notable that two independent groups have confirmed the association of Rab18 with lipid droplets [72,73]. Both TAG droplets and aqueous cored vesicles are transported on microtubules [10,74–76]. ARF1 and PLD regulate the recruitment of the trafficking coat proteins to aqueous cored vesicles [77,78], and also regulate binding of adipophilin to TAG droplets [79–81]. These data add to the emerging model that TAG droplets and aqueous-cored vesicles share trafficking machinery.

9. Evolution of the PAT family

In this review we have proposed that EPATs (TIP47, S3–12, and OXPAT) evolved to maximize storage of excess feeding-derived energy as TAG in lipid droplets and that CPATs evolved to manage the TAG energy store in response to energy demands. The sequence similarities, lipid binding characteristics, and chromosomal locations of genes encoding the PAT proteins point to a common ancestral gene. In mice and humans the EPAT genes lie within ~200 kilobases of one another with the OXPAT and S3–12 genes being contiguous, which suggests that two of the EPAT genes arose by gene duplication. We speculate that one of the EPATs was the ancestral PAT gene, because the EPATs are stable in the absence of lipid, and thereby are a readily available pool of coat proteins to accommodate efficient TAG packaging in response to acute lipid loads. CPATs must bind neutral lipid for stability, so CPATs cannot form a cytosolic reservoir to facilitate rapid TAG packaging.

At least two sequence based evolutionary analyses of the PAT family have been published [23,27]. Both of these analyses show that adipophilin and TIP47 are the least divergent members of the PAT family. Consistent with these observations, adipophilin and TIP47 are almost ubiquitously expressed in mammalian cells. For these reasons, it is likely that one of these proteins represents the ancestral gene. The observations that TIP47 is ubiquitously expressed, is an EPAT, and is the only PAT gene that is not PPAR regulated, suggest that it is a housekeeping gene and likely gave rise to the more specialized PAT proteins. Hence, a common ancestral gene was likely duplicated four times and the CPATs, perilipin and adipophilin, migrated to different chromosomes.

10. Standing PAT but looking forward

Animals have evolved multiple lipid droplet structural proteins to accommodate both fasting and feeding and to manage triacylglycerol stores. These PAT family proteins share sequence similarity and motifs for lipid droplet binding but differ in their tissue expression, stability in the absence of neutral lipid, and lipid droplet localization during rapid fatty acid flux into cells. We have proposed that the EPATs, the exchangeable TAG-associating PAT proteins (TIP47, S2–12, and OXPAT), function as a preexisting reservoir of lipid droplet coat protein to package new TAG for delivery to storage droplets. These storage droplets are coated by the CPATs, the constitutive TAG-associating PAT proteins (adipophilin and perilipin), which control access of lipases to the TAG store.

A major question now before us is what are the specific functions that each PAT protein has evolved to perform. Why do some tissues need all three EPATs and others only one? Some answers to these questions will come from careful analysis of gain and loss of function experiments in genetically engineered mice. Gene knockouts of the CPATs have been highly informative; in particular, the perilipin knockouts independently reported by the Londos and Chan labs have led to a paradigm shift in our understanding of perilipin not just as a barrier to lipases but also as a facilitator of hormone responsive lipase action in adipocytes.

Additional insights have and will continue to come from the complete description of the lipid droplet proteome in different tissues, under different metabolic states, and in response to different pharmacological treatments. The hydrophobicity and low abundance of many lipid droplet proteins present challenges, but proteomic approaches are rapidly adapting to improve sensitivity for such proteins. The identification of protein complexes by mass spectrometry may reveal unanticipated networks of regulation between lipid droplets and other organelles and pathways.

How will the study of lipid droplets and the proteins that coat them translate into knowledge pertinent to human health and disease? Failure to package fatty acids as TAG in lipid droplets likely contributes to the damage associated with diabetes and obesity [2,82,83], hence failure to effectively package fatty acids takes a large and growing human and economic toll. The role of PAT proteins in controlling fatty acid flux both within cells and between the adipose depot and peripheral tissues suggests them as candidates for genetic polymorphisms that contribute to abnormal lipid storage and metabolic dysfunction. All PAT proteins, except TIP47, are already drug targets in that their expression is induced by PPAR activation in specific mouse tissues and cultured cells. It will be interesting to determine if the PATs are similarly regulated by PPARs in human tissues. This minireview has discussed the role of EPATs in packaging newly synthesized TAG and delivering it to the CPAT storage compartment. Given this role, it is possible that pharmacological manipulation of EPATs may be exploited to promote appropriate lipid packaging and, thereby, limit lipotoxicity in vulnerable peripheral tissues, such as striated muscle, the vasculature, liver, and the endocrine pancreas.

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