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REVIEW

Molecular mechanisms of perilipin protein function in lipid droplet metabolism

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Perilipins are abundant lipid droplet (LD) proteins present in all metazoans and also in Amoebozoa and fungi. Humans express five perilipins, which share a similar domain organization: an amino-terminal PAT domain and an 11-mer repeat region, which can fold into amphipathic helices that interact with LDs, followed by a structured carboxy-terminal domain. Variations of this organization that arose during vertebrate evolution allow for functional specialization between perilipins in relation to the metabolic needs of different tissues. We discuss how different features of perilipins influence their interaction with LDs and their cellular targeting. PLIN1 and PLIN5 play a direct role in lipolysis by regulating the recruitment of lipases to LDs and LD interaction with mitochondria. Other perilipins, particularly PLIN2, appear to protect LDs from lipolysis, but the molecular mechanism is not clear. PLIN4 stands out with its long repetitive region, whereas PLIN3 is most widely expressed and is used as a nascent LD marker. Finally, we discuss the genetic variability in perilipins in connection with metabolic disease, prominent for PLIN1 and PLIN4, underlying the importance of understanding the molecular function of perilipins.

Keywords: amphipathic helix; lipase; lipid droplet; lipodystrophy; lipolysis; metabolic disease; perilipin; perilipin phylogeny

Perilipins are a family of evolutionarily conserved and abundant lipid droplet (LD) surface proteins. In proteomics studies of LDs isolated from different human or mouse tissues, one or more perilipins are generally found to be among the most abundant LD-associated

proteins [1–6]. Their expression is highly regulated by cellular developmental programs or external factors, such as attacks by pathogens, and they can serve as convenient markers to follow LDs or cell differentiation [7–10]. The defining member, perilipin 1 (PLIN1),

Abbreviations

aa, amino acid; AH, amphipathic helix; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; CMA, chaperone-mediated autophagy; C-ter, carboxy-terminal; DAG, diacylglycerol; DGAT2, Diacylglycerol O-Acyltransferase 2; ER, endoplasmic reticulum; ESC, embryonic stem cells; FA, fatty acid; GPAT4, glycerol-3-phosphate acyltransferase 4; GWAS, genome-wide association study; HFD, high-fat diet; HSL, hormone-sensitive lipase; KO, knock-out; LD, lipid droplet; MAG, monoacylglycerol; MGL, monoacylglycerol lipase; NAFLD, nonalcoholic fatty liver disease; NBD, nitrobenzofurazan; N-ter, amino-terminal; PKA, cAMP-dependent protein kinase; PLIN, perilipin; pLoF, predicted loss of function; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; SNV, single nucleotide variant; T2D, type 2 diabetes; TAG, triacylglycerol; VLDL, very low-density lipoprotein particle; WAT, white adipose tissue; WES, whole exome sequencing; WHR, waist-to-hip ratio.

was first identified in 1990 as the most highly phosphorylated protein in lipolytically active human adipocyte cells [11]. It was later shown to specifically localize to the surface of LDs, both in adipocytes and when heterologously expressed in other cell types, hence the name perilipin, derived from Greek for ‘surrounding lipid’ [12–15]. In the following years, four additional members of the family were identified in human and mouse tissues, now named perilipin 2–5 (PLIN2–PLIN5) [16–21]. All perilipins primarily localize to LDs and are generally thought to function on the LD surface, despite an initial disagreement in the case of PLIN3, which was identified as an endosomal trafficking protein [18], whereas PLIN4 was initially identified through its association with the plasma membrane of adipocyte cells [19].

The perilipin family was first defined by homology in the amino-terminal (N-ter) region, which was termed the PAT domain (for ‘perilipin, adipophilin, TIP-47’; the original names for PLIN1, PLIN2, and PLIN3, respectively) [22]. The PAT homology domain is also present in PLIN5; in contrast, the N-ter region is shorter in PLIN4 and lacks significant homology

with other perilipins (Fig. 1). Following the PAT domain, another feature shared by all perilipins is a region of 11-amino acid (aa) (11-mer) repeats, which varies in length between ~ 5 repeats in PLIN5 and 7–9 repeats in PLIN1–PLIN3, whereas it is extended to ~ 90 repeats in the canonical human form of PLIN4 [23–25]. Finally, the perilipins are also related *via* their carboxy-terminal (C-ter) domain, which has been shown to fold into a 4-helix bundle in PLIN3 [26], and is well-conserved in other perilipins, except for PLIN1 (Fig. 1). Structural and biochemical features of perilipins and their cellular targeting are discussed in more detail below.

In addition to sequence- and structure-based homologies, notable in Fig. 1 are also differences between the five perilipins; e.g., the divergence in the C-ter region of PLIN1; the highly extended repetitive region in PLIN4; the presence of a mitochondria-interacting sequence in PLIN5 [27]. These differences immediately suggest that the different perilipins may have adapted for some specialized functions. Perilipins also vary in terms of their tissue expression, with the two more canonical-looking perilipins, PLIN2 and PLIN3, showing a ubiquitous expression

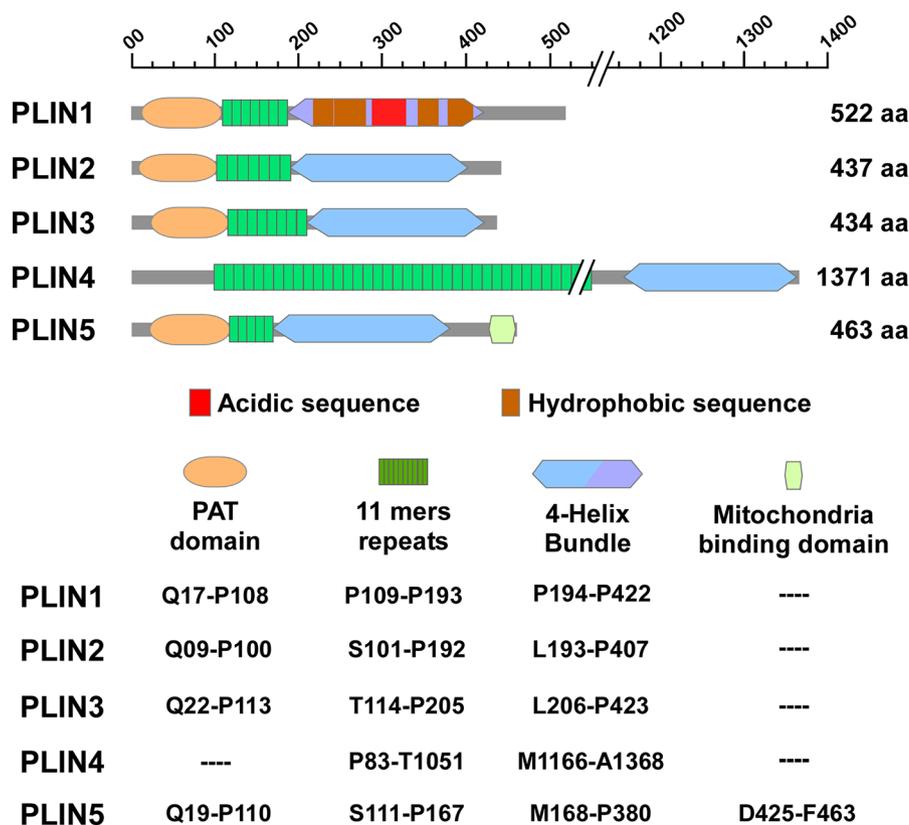


Fig. 1. Comparison of domain structure of the human perilipins. The canonical form of each perilipins is shown with the first and last residue of each domain indicated.

pattern, whereas PLIN1 and PLIN4 are highly expressed in adipocyte and steroidogenic cells, with their levels increasing during adipocyte differentiation. The function of PLIN1 in adipocyte cells has been the most extensively studied. Recent work has identified subpopulations of adipocyte cells that differ in their perilipin expression levels, with only Adipo^{PLIN} (adipocytes with high perilipin expression) showing a response to insulin, suggesting functional specialization between different adipocyte cells [28]. PLIN4 is also highly expressed in muscles, and PLIN5 is expressed in oxidative tissues such as muscle and brown adipocyte tissue [20].

Perilipins are not known to possess any enzymatic function. However, they play an important role in the regulation of lipase activity on the LD surface. This function has been most studied for PLIN1, and also for PLIN5, but it is not clear if it is shared by all members of the family (see below). In addition to their interaction with lipolytic enzymes and regulators of lipolytic activity on the LD surface, perilipins also interact with other LD-regulating proteins and might participate in LD biogenesis, in physical regulation of the LD surface or in fatty acid (FA) channeling to other compartments. Non-lipolytic functions of perilipins will be discussed later. Finally, the importance of perilipins for human metabolism is underscored by the strong association between genetic variability in the perilipin genes, particularly in *PLIN1* and *PLIN4*, and metabolic disease, as discussed in the final section of this review.

Since their discovery, perilipins have been the subject of many investigations, and it would be difficult to cover all work on perilipins in a single Review. For the physiological and pathological roles of perilipins in different tissues, and their transcriptional regulation, we refer readers to recent reviews that have covered these topics extensively [29,30]. Here, we will focus on the underlying molecular mechanisms of perilipin function, specifically for the human and mouse proteins. However, it is useful to first make a short detour into the phylogeny of the perilipin family to define its family and how the different perilipins relate to one another.

Phylogenetic analysis of perilipins

Most organisms have developed mechanisms to store lipids in LDs, primarily as a source of energy [31]. Even though phospholipids are sufficient for separating the hydrophobic neutral lipids from the surrounding cytosol, LD proteins play a crucial role in the homeostasis of LDs. The Londos and Kimmel group were the first to propose that perilipins have a distant ancestral origin, identifying two perilipin homologs,

lipid storage droplets surface-binding protein 1 and 2 (LSD-1 and LSD-2, respectively) in *Drosophila melanogaster*, and a more distant homolog in the social amoeba *Dictyostelium* [22,32]. This suggests that the perilipin gene appeared before the split of metazoans within the Unikonta group (which groups Amoebozoa and Opisthokonta, the latter containing Metazoa and Fungi), as confirmed by subsequent phylogenetic analyses [29,33] (Fig. 2). Although the sequence similarity between the dictyostelium and human perilipins is not very high (18% with human PLIN3), the structures of these proteins, as predicted by AlphaFold2, contain both an N-ter triangular helical PAT domain and a C-ter 4-helix bundle (Fig. 3).

Surprisingly, a perilipin gene was not detected in the budding yeast *Saccharomyces cerevisiae* nor in the nematode *Caenorhabditis elegans* [22]. Since then, other groups have identified perilipin-like proteins in *C. elegans* as well as in fungi [34–37]. In *S. cerevisiae*, two proteins, Sps4 and Pet10p (also known as Pln1), exhibit similarities with the N-ter perilipin PAT domain, and Pet10p is found to be abundant on LDs [35,37,38]. However, the structural resemblance of the C-ter region is far less obvious, and, if these proteins are related to the metazoan perilipins, they have substantially diverged. By contrast, from a predicted structural point of view, more canonical perilipin-like proteins appear to exist in several, if not all, major subkingdoms of fungi, including Dikarya (Basidiomycota and Ascomycota) and other fungi [39] (Fig. 2).

Whole-genome duplication events in early vertebrates [vertebrate genome duplication (VGD)1 and VGD2] and in teleostei [teleost-specific genome duplication (TGD)], along with the specialization in multi-tissue organisms, can explain why most vertebrates express multiple perilipins. Consecutive gene duplication events (exemplified by human *PLIN3*, *PLIN4*, and *PLIN5*, all located within 400 kbp on Chr19p13–3, with *PLIN4* and *PLIN5* being separated by ~ 4 kb) likely contributed to the divergence of the perilipin family and the specialization of perilipin protein functions in higher organisms [33]. These fairly recent duplication events are illustrated by the presence of a common phylogenetic clade containing *PLIN3–PLIN5* (Fig. 2). *PLIN4*, characterized by a long extension of the central repetitive region, is only present in mammals and is not found in birds. However, three perilipin genes are present on Chr28 in chicken, of which two are adjacent. This suggests that the gene duplication occurred earlier in the vertebrate evolution, before the expansion of the central repeats.

In ray-finned fish (Actinopterygii), a specialized perilipin (Plin6) targets the surface of carotenoid droplets

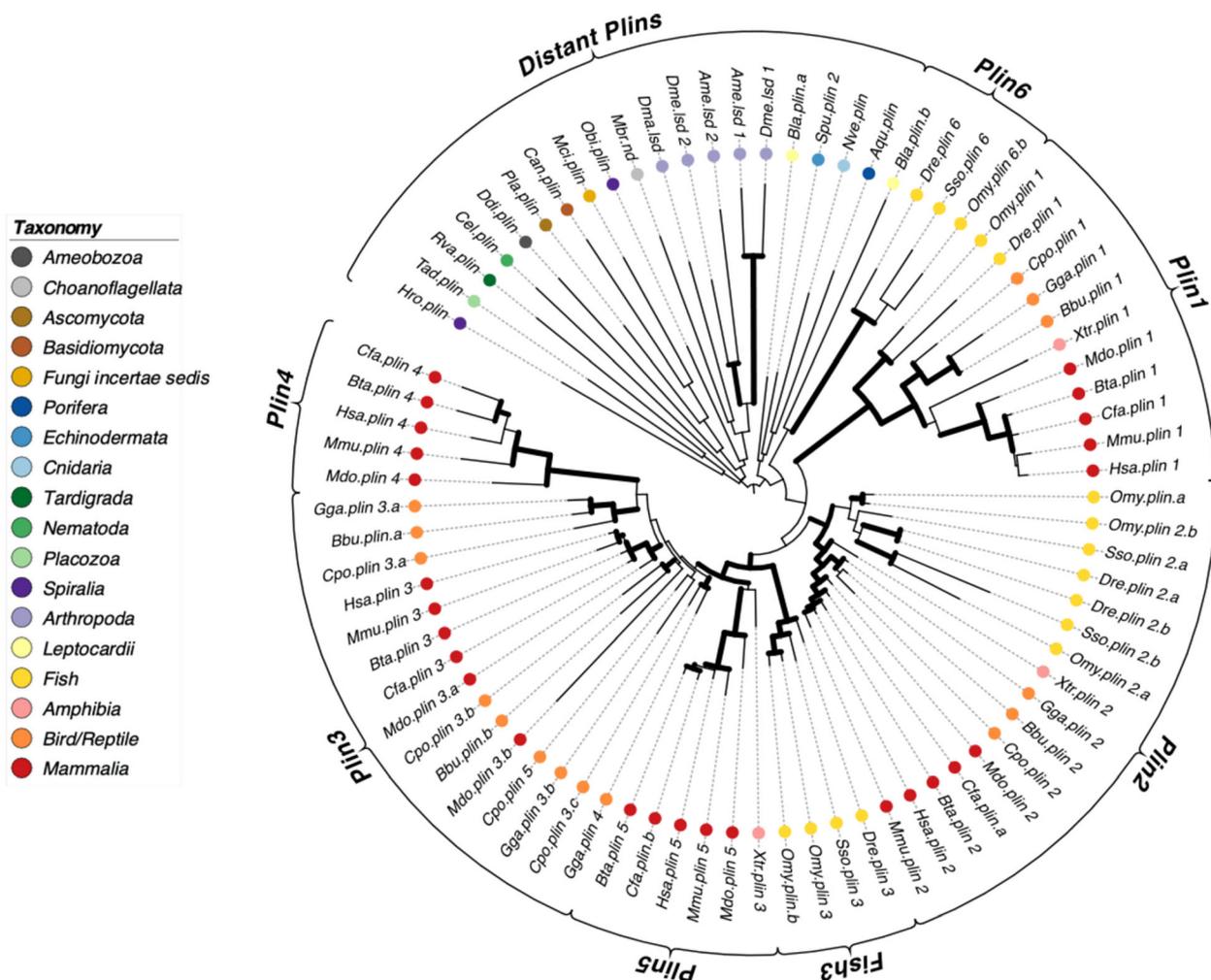


Fig. 2. Phylogenetic analysis of perilipins. Human PLIN1 (O60240) was used as a query with SHOOT.bio [237] using the Metazoa database to extract a phylogenetic tree consisting of 196 sequences from 46 organisms. A subset of 22 representative species was selected, and an additional 7 perilipins from other organisms were added (labeled * below). Species were from the following taxa, as defined by TaxonKit [238]. Kingdoms are shown in bold, and classes are underlined. Metazoa: Leptocardii: *Branchiostoma lanceolatum* (Bla); Mammalia: *Homo sapiens* (Hsa), *Mus musculus* (Mmu), *Canis familiaris* (Cfa), *Bos taurus* (Bta), *Monodelphis domestica* (Mdo); Actinopterygii (Fish): *Danio rerio* (Dre), *Oncorhynchus mykiss* (Omy), *Solea solea* (Sso); Archelosauria (Bird/Reptile): *Crocodylus porosus* (Cpo), *Bubo bubo* (Bbu), *Gallus gallus* (Gga); Amphibia: *Xenopus tropicalis* (Xtr); Arthropoda: *Daphnia magna* (Dma), *Drosophila melanogaster* (Dme), *Apis mellifera* (Ame); Nematoda: *Caenorhabditis elegans** (Cel); Tardigrada: *Ramazzottius varieornatus** (Rva); Cnidaria: *Nematostella vectensis* (Nve); Echinodermata: *Strongylocentrotus purpuratus** (Spu); Porifera: *Amphimedon queenslandica* (Aqu); Placozoa: *Trichoplax adhaerens* (Tad); Spiralia: *Helobdella robusta* (Hro), *Octopus bimaculoides* (Obi); Fungi: Basidiomycota: *Cantharellus anzutake** (Can); Ascomycota: *Protomyces lactucae-debilis** (Pla); Fungi incertae sedis: *Mucor circinelloides** (Mci); Amoebozoa: *Dictyostelium discoideum** (Ddi); Choanoflagellata: *Monosiga brevicollis* (Mbr). Muscle5 [239] was used to generate a diversified ensemble, resampled with a minconf of 0% to retain a complete alignment. FastTree 2 [240] was used to generate 100 trees from the 100 resampled alignments and the newick conf command was used to generate the final tree file with bootstrap confidence values. The tree (midpoint rooted) and custom coloring annotations, based on the organism taxonomy, were rendered using the iTOL website [241]. Nodes with bootstrap values above 70 (out of 100) are shown as thicker lines (see Table S1 for details).

in the skin, especially in well-pigmented species [33]. It is difficult to determine the exact appearance of Plin6 in evolution, but the presence of a Plin6-like protein in the lancelet *Branchiostoma lanceolatum* (Bla, Fig. 2) suggests that this event occurred before the appearance

of Craniata, but was lost in terrestrial species. However, it cannot be excluded that this is the result of a later parallel or convergent evolution. Due to the TGD, most fish genomes contain far more than six perilipin-like genes (e.g., *Dra.plin 2a* and *Dra.plin 2b*,

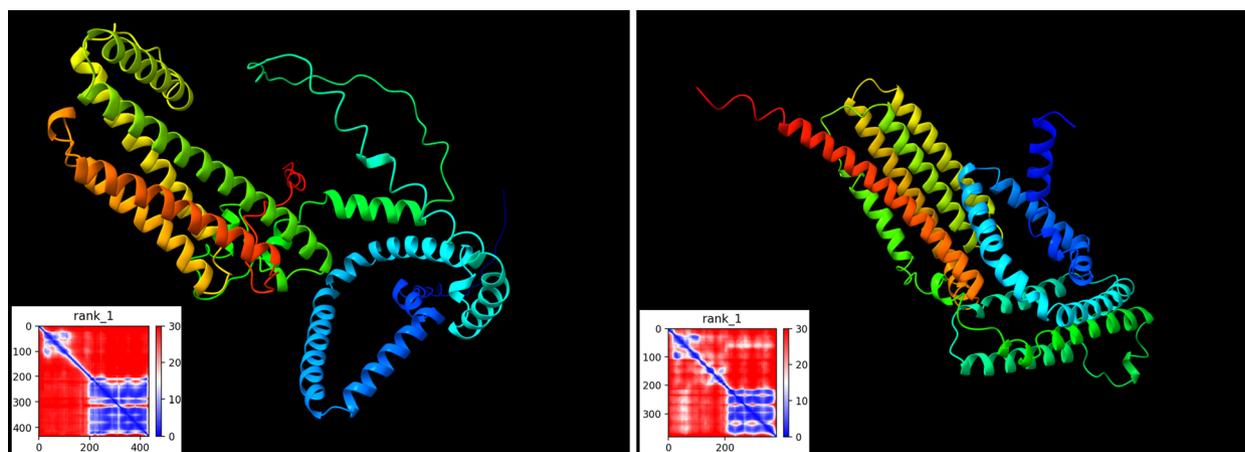


Fig. 3. Comparison of predicted structures of human PLIN3 and dictyostelium perilipin. Rainbow-colored (from N-ter in blue to C-ter in red) AlphaFold2 [242] predicted structures of human PLIN3 (left) and perilipin from the amoeba *Dictyostelium discoideum* (right). Despite low overall sequence conservation (18% identity), both proteins are predicted to form a very similar structure, with an N-ter PAT domain consisting of three α -helices forming a triangular structure (in blue) and a C-ter 4-helix bundle. The predicted aligned error (insert) indicates regions that likely fold into separate domains (in blue), with the N-ter and C-ter in the upper left and lower right corner, respectively. The different orientation of the PAT domain in the two structures is likely due to the structural unpredictability of the central sequence (in light blue/dark green).

Fig. 2). Interestingly, we note that *plin1* has been lost in fish with low lipid content, such as sole and cod (and could not be found in any Neoteleostei fish), but is present in lipid-rich species, such as herring, trout, and salmon. Overall, phylogenetic analysis of perilipins offers an interesting perspective on their functional specialization.

Structural and biochemical properties of perilipins

Structures of full-length perilipins have so far not been experimentally determined, due to difficulties associated with studying multi-domain peripheral membrane proteins. Although recent advances in structure prediction (e.g., AlphaFold) offer insight into what full-length perilipins might look like (Fig. 3), caution is needed as these structures represent proteins in solution, whereas the LD-bound structures are likely highly dependent on extended interactions with the LD surface.

A number of studies have focused on the structures and behavior of different perilipin segments and domains. The N-termini of all mammalian perilipins except PLIN4 show the highest level of sequence similarity within the perilipin sequence (40–60% sequence identity in the human proteins) and were predicted to form α -helices, leading to the definition of the N-ter PAT domain [22,40]. However, the function of the PAT domain has remained elusive. Early work showed

that the PAT domain of PLIN1–PLIN3 was neither sufficient nor required for LD binding in cells [41–45]. The Pfeffer group has proposed that the PAT domain of PLIN3 functions as an oligomerization domain, based on their observations that purified PLIN3 and its N-ter 133-aa fragment eluted from a size-exclusion column at more than 6 or more than 20 times their expected molecular weights, respectively, whereas combining the two species led to the formation of even larger protein complexes or aggregates [46]. The importance of this oligomerization for PLIN3 LD binding or function has not been evaluated.

A recent study investigated the structure and the functional role of the PAT domain in PLIN3 [47]. Based on structure predictions and on hydrogen–deuterium exchange mass spectrometry analysis of PLIN3 upon membrane binding, the authors propose a structural transition from disordered to helical of a region comprising the PAT domain and the downstream 11-mer repeats. The PAT domain itself forms a stable triangular helical structure and displays a high affinity for diacylglycerol (DAG)-enriched phospholipid bilayers, leading to the proposal that its function is to recognize DAG in nascent LDs forming at the endoplasmic reticulum (ER) and to promote the binding of the 11-mer repeat region to the phospholipid monolayer. DAG was previously shown to be important for PLIN3 binding to membranes and LDs [48], but it is not known whether this is also the case for other perilipins. Heterologous expression of human PLIN3 in budding yeast confirms its sensitivity to

DAG levels in the ER and suggests that PLIN3 could promote the formation of DAG-enriched membrane domains, although in this case the PAT domain was not sufficient for DAG enrichment, which required expression of at least two of the three PLIN3 domains in tandem [49]. Based on these observations and the fact that DAG is a precursor in the synthesis of triacylglycerol (TAG), which forms the core of LDs, it has been proposed that PLIN3 may play a role in LD biogenesis from the ER membrane. This hypothesis has not been directly evaluated and the function of PAT domains of different perilipins awaits further investigation.

Following the PAT domain, all perilipins contain a region of 11-aa repeats of variable length (Fig. 1). These sequences are predicted to be disordered in solution and have been shown to fold into amphipathic helices (AHs) in the presence of a lipid surface [24,25]. They are also sufficient to target LDs in a model cell when expressed by themselves, and increased length promotes their interaction with LDs, as has been demonstrated with the PLIN4 sequence [25,50]. These observations are consistent with earlier studies showing that N-ter regions of PLIN1–PLIN3, comprising the 11-mer repeats, target LDs in cells [24,41,45,51,52]. In the case of PLIN5, which contains only five 11-mer repeats, the PAT domain and the 11-mer region are together required for efficient LD targeting [27,53].

The presence of 11-mer repeats suggests that these regions could fold into a so-called 3–11 helix, which is a slightly extended version of the standard α -helix [54]. These types of helices are quite rare and have been structurally characterized in only two unrelated groups of proteins, in α -synuclein and in apolipoproteins, where they have been shown to laterally interact with the lipid surface [23,55,56]. It is not known what advantage a 3–11 helix might have over a standard α -helix, and, given the subtle differences between the two types of helices, this question is quite difficult to address. A recent study identified a putative 3–11 helix in another LD protein, spartin, which has been shown to compete with PLIN3 for binding to the LD surface [57].

Interestingly, a strong compositional bias exists in the 11-mer regions of perilipins, which is most prominent in PLIN4: its entire repetitive sequence of close to 1000 aa-s is almost entirely devoid of bulky hydrophobic residues but is enriched in the small neutral-hydrophobic residues valine (V), glycine (G) and threonine (T) [25]. The absence of large hydrophobic residues, also seen in other perilipins [50], is a feature that promotes LD specificity. Mutations that only slightly increase the hydrophobicity do not affect AH binding to LDs, but they make the AH more

promiscuous, invading bilayer membranes [25]. This finding can be explained by considering the properties of the LD surface, which is covered by a phospholipid monolayer and whose surface tension can vary depending on the density of phospholipid coverage [58]. *In silico* studies using molecular dynamics simulations suggest that increased surface tension promotes AH targeting to LDs, making this a prominent parameter that promotes selectivity in LD targeting [59,60]. The central repetitive regions of perilipins may therefore have been selected for weak interaction with the LD surface, allowing them to interact with LDs specifically and in a reversible manner over a large surface area [50,61]. This may be particularly important for maintaining LD stability and surface-to-volume ratio. We have demonstrated that the PLIN4 AH can replace the phospholipids on LDs *in vitro* and in a model cell system, creating a uniform and stable surface that prevents LD fusion or clustering [25]. Furthermore, polar or charged residues in PLIN4 may mediate intermolecular interactions between adjacent helices that would promote assembly of a PLIN4 coat on the LD surface [50].

With regard to the C-ter segments of mammalian perilipins, the structure of the mouse PLIN3 C-ter domain has been resolved by X-ray crystallography [26] and resembles the 4-helix bundle domain found in apolipoproteins, although there is no sequence homology. In apolipoproteins, this domain promotes reversible binding to small lipid particles *via* conformational switching between helix–helix or helix–lipid interactions [56]. It has been proposed that such conformational switching could also take place in perilipins [52], but this possibility remains to be explored experimentally. HHPred analysis [62] of PLIN2, 3, 4, and 5 sequences, possessing considerable sequence similarity (from 45 to 61%), suggests that these proteins also contain 4-helix bundle structural motifs. This prediction is supported by comparison of purified C-ter from PLIN2 and PLIN3 by limited proteolysis [63]. AlphaFold models of the different human perilipins (PLIN1: AF-O60240-F1, PLIN2: AF-Q99541-F1, PLIN3: AF-O60664-F1, PLIN4: AF-Q96Q06-F1, PLIN5: AF-Q00G26-F1) agree with this prediction in all perilipins except for PLIN1, whose C-ter sequence is more divergent (29–35% sequence similarity), and seems less compatible with a 4-helix bundle structure. Analysis of the corresponding region in PLIN1, including the 11-mer repeat region (P114–P422), has identified three hydrophobic segments surrounding an acidic region, which are important for PLIN1 binding to LD [42,64]. The mode of interaction of this region of PLIN1 with LDs has been recently explored by Klemm and

coworkers. They show that the first hydrophobic segment mediates high lipid-binding affinity of PLIN1, possibly acting as a hairpin loop to anchor PLIN1 to the LD surface or the ER bilayer, with an additional contribution of a downstream hydrophobic region [65].

The C-ter regions of PLIN3, PLIN4, and PLIN5 do not appear to directly contribute to LD binding in cells because they remain cytosolic when expressed on their own [25,45,47,52,53]. In contrast, a region within the 4-helix bundle of PLIN2 appears to contribute to LD binding [43,51,52], which could explain why, compared to PLIN3 and PLIN4, PLIN2 is more stably associated with LDs in cells (see below). For PLIN3, the Kooijman group observed, using a pendant drop tensiometry system, that both the full-length protein or its 4-helix bundle were able to bind to the oil droplet when it was not fully covered with phospholipids, meaning that it had an increased surface tension [66]. These experiments also showed some preference of full-length PLIN3 for mono-unsaturated phosphatidylcholine or for phosphatidylethanolamine in the case of its 4-helix bundle. Whether perilipins display some phospholipid preferences in cells is not known.

A lipid-binding pocket, named the hydrophobic cleft, has been identified within the PLIN3 4-helix bundle domain [26], with a highly conserved sequence (PL/VxWLVGPF) present in most perilipins, except for PLIN1. The presence of this cleft as a conserved structural feature suggests that it is functionally important. Direct NBD-stearate binding was described with purified PLIN2 [67]. A conserved tryptophan exposed at the bottom of the PLIN2 pocket enabled the demonstration of its direct interaction with lipids by tryptophan fluorescence quenching [68]. Modeling showed possible accommodation of either cholesterol or stearic acid in the hydrophobic cleft [68]. More recently, specific binding of mono-unsaturated FAs in the PLIN5 lipid-binding pocket has been demonstrated and linked to a putative signaling function of PLIN5 [69].

In addition to the three segments common to all mammalian perilipins, PLIN1 and PLIN5 contain unstructured C-ter extensions that mediate specific protein–protein interactions, important for regulation of lipolysis in the case of PLIN1 [70–72], or interaction between LDs and mitochondria in the case of PLIN5 [27,73]. Three additional isoforms of PLIN1 have been identified in mice: PLIN1B, present in adipocytes, is truncated after the 4-helix bundle region (measuring 422 aa, compared to 517 aa for murine PLIN1A), and the shorter isoforms PLIN1C and PLIN1D (347 and 244 aa, respectively), which lack the 4-helix bundle

region and are expressed in steroidogenic cells [22]. The functions of C-ter tails of PLIN1 and PLIN5 are further discussed in later sections.

Targeting of perilipins to LDs in cells

In addition to considerable differences in their domain organization and tissue expression, perilipins also differ in their interaction with LDs and have been divided into two groups: constitutive perilipins and exchangeable perilipins [74]. Constitutive perilipins, namely PLIN1 and PLIN2, are always observed on LDs, require LDs for their stability, and are degraded in their absence. PLIN1 is mainly expressed in white adipose tissue (WAT) and is detectable on LDs after several days of adipocyte differentiation of mouse 3 T3-L1 cells [17,75,76] or human [77] or mouse [78] stem cells. In cultured steroidogenic Y-1 adrenal cortical cells, which also express endogenous PLIN1 [7], PLIN1 levels highly increase upon addition of oleic acid and cholesterol, correlating with LD abundance. This is likely due to an increase in PLIN1 protein stability [7,79]. PLIN2 levels are likewise increased when cells are cultured in the presence of oleic acid, whereas, upon LD depletion, the protein is unstable in the cytoplasm and is subject to proteasomal degradation, which may be initiated by PLIN2 N-ter acetylation [44,78,80]. Consistently, PLIN2 is found in LD fractions by cell fractionation [17,75,76]. PLIN2 is also degraded by chaperone-mediated autophagy (CMA), which facilitates lipolysis [81]. In C2C12 cultured myocytes, PLIN2 levels correlate with LD abundance, whereas depletion of PLIN2 enhances LD size and lipolysis, suggesting reciprocal stabilization between LDs and PLIN2 [82]. To study this relationship, the Olzmann group [83] generated genome-edited liver cell lines in which PLIN2 was endogenously labeled with GFP and performed an extensive CRISPR-Cas9 screening to identify proteins that affect the number and stability of PLIN2-decorated LDs. This approach identified known and new factors that regulate PLIN2 abundance under different metabolic conditions and confirmed the stable association of PLIN2 with LDs.

In contrast to PLIN1 and PLIN2, the exchangeable perilipins PLIN3, PLIN4, and PLIN5 are stable in the cytosol [45,75,84], but can relocate to the LD surface under lipogenic conditions. Protein synthesis is not required for their localization to LDs in 3 T3-L1 mouse adipocyte cells [75]. Endogenous PLIN3 and PLIN4 localize to LDs shortly (within minutes) after treatment with oleic acid to induce LD formation [45,85]. PLIN3 is used as a marker to detect small/nascent LDs [86]. As mentioned above, PLIN3 was

also shown to bind to DAG in the ER bilayer. PLIN5, which contains a mitochondria-targeting sequence, can be observed in the cytosol, on mitochondria and at LD–mitochondria contact sites. The latter appears as puncta on LDs by super-resolution microscopy and do not colocalize with PLIN2 puncta [87].

The distinction between constitutive and exchangeable perilipins has been widely adopted by the research community. However, this distinction is not always clear-cut and the underlying molecular mechanisms remain a matter of investigation. Notably, PLIN2 displays intermediate features between constitutive perilipins and exchangeable perilipins. Tagged PLIN2 has been observed in the cytosol in a few studies [52,88]. In fluorescence recovery after photobleaching (FRAP) experiments on heterologously expressed PLIN1, PLIN2, and PLIN5, the proteins displayed similar lateral mobilities on LDs, but PLIN2 and PLIN5 exchanged between the cytoplasm and the LDs, whereas PLIN1 remained bound to LDs [89]. Importantly, PLIN1 binding to LDs is observed both under basal and lipolytically stimulated conditions [90]. In addition, some PLIN1-associated ER signal has been observed in OP9 adipocyte cells for the endogenous protein by immunofluorescence and cell fractionation [48,91], as well as for heterologously expressed GFP–PLIN1 in liver-derived Huh7 cells [52].

In most general terms, two pathways for protein targeting to LDs have been proposed: the ERTOLD and the CYTOLD pathway [92]. The ERTOLD pathway pertains to proteins (also termed class I LD proteins) that relocate from the ER, the site of LD biogenesis, to the LD surface. A number of these proteins have been shown to contain an extended transmembrane-like helix, which could adopt a hairpin loop in the ER membrane and thus relocate to the phospholipid monolayer of the budding LD, where the environment would be energetically more favorable [93,94]. Reference proteins of this group are glycerol-3-phosphate acyltransferase 4 (GPAT4) and diacylglycerol O-acyltransferase 2 (DGAT2), enzymes involved in the synthesis of TAGs [95,96]. ERTOLD proteins can also take advantage of the physical continuity between LDs and the ER or rely on membrane-fusion machinery to form new membrane bridges between the cytosolic LDs and the ER membrane. Skinner *et al.* [91] have proposed that PLIN1 moves from the ER to LDs during lipid synthesis. In agreement, a recent preprint shows that PLIN1 can interact with the ER and LDs using hydrophobic segments in its degenerate C-ter region (Fig. 1), suggesting that PLIN1 targets LDs *via* the ERTOLD pathway [65].

A recent study by the Walther and Farese group explored ERTOLD targeting in a *Drosophila* cellular

model, identifying at least two distinct groups of ERTOLD proteins, early and late [97]. Although the early group is presumed to target LDs during LD biogenesis, the late group requires protein machinery that is not involved in LD budding; for example, the COPII proteins involved in vesicular transport from the ER. Earlier studies have implicated the retrograde COPI vesicular transport in targeting of patatin-like phospholipase domain-containing protein 2 (ATGL) or GPAT4 and DGAT2 to the LD surface in mammalian and *Drosophila* cells [98–100]. The details of this pathway remain to be elucidated. Interestingly, knock-down of components of COPI and COPII complexes, or overexpression of dominant-negative mutants, also strongly decreased the localization of PLIN2, but not of PLIN3, to LDs [99,101]. COPI vesicle budding from LDs has been suggested to decrease phospholipid density on the LD surface [102], which could provide more favorable conditions for PLIN2 binding, but these effects will need to be further explored.

CYTOLD, or class II LD proteins, are recruited to LDs from the cytosol and in many cases interact with the lipid surface of LDs utilizing AHs [55,61,103,104]. As discussed above, all perilipins contain 11-mer repeat regions of significant lengths that can fold into AHs and contribute to their LD targeting. In addition, the 4-helix bundle regions could also open up in some cases, revealing additional AHs that could interact with LDs [52]. Because AHs bind laterally to the lipid surface, and are very long in perilipins compared to other AH-containing proteins [24,50,103], they would occupy a relatively large surface area, resulting in a significant influence of protein crowding on perilipin LD targeting [52,105]. What assures the specificity of perilipin AH targeting to LDs? Prévost *et al.* [106] have proposed that bulky hydrophobic residues are important for AH targeting to LDs, recognizing phospholipid packing defects on the LD surface. However, this conclusion is difficult to reconcile with the fact that large hydrophobic residues are present in many AHs that target the ER, the nuclear envelope, or the Golgi membrane, inserting into large packing defects present in these bilayers [61,103,104]. In fact, an important adaptation of the perilipin central AH regions is being almost completely devoid of large hydrophobic residues, decreasing their affinity for bilayers while still being able to insert into the LD monolayer [25,50]. Surface tension is a parameter that can vary in LD lipid monolayers, but only negligibly in lipid bilayers [58,59,107]. Increased surface tension of LDs may be an important parameter for the selective recruitment of perilipins *via* their AHs, which would imply that their binding to LDs is coupled with larger

perturbations of the LD surface (LD growth, onset of lipolysis, etc.).

Despite their general classification into two groups, the mechanisms of perilipin targeting to LDs in cells are very complex, due to several factors:

- 1 perilipins can utilize different domains for LD targeting, which can act in concerted or opposite manners, likely differing between different perilipins;
- 2 their targeting to LDs is regulated transcriptionally and post-translationally, with post-translational modifications leading to changes in their LD surface affinity or promoting their degradation;
- 3 the amount of available LD surface in the cell at a given time is finite and is influenced by lipogenesis, lipolysis, and perilipins themselves, which have been shown to stabilize LDs and also depend on LDs for their stability, leading to non-linear effects;
- 4 perilipins have been suggested to self-assemble, which would again result in non-linear effects;
- 5 perilipins may be sensitive to the LD lipid composition, and, because LD composition differs between tissues and metabolic conditions, targeting of perilipins to LDs may be cell-type specific.

A striking observation is that, within the same cell, different perilipins can be observed decorating different LDs [75,108,109]. Based on their results in cultured adipocyte cells, Wolins *et al.* [74] proposed that perilipins bind to LDs in a hierarchical manner, with PLIN3 and PLIN4 binding to nascent LDs, formed after addition of oleic acid, PLIN2 to intermediate LDs, and PLIN1 to large LDs present in mature adipocytes. This model follows an earlier immunofluorescence analysis in 3 T3-L1 mouse cultured adipocytes by Brasaemle *et al.* [17], who observed that PLIN2 localized to LDs early during differentiation and was later entirely replaced by PLIN1, with only a small overlap between the two proteins between day 3 and day 5. This correlated with an increase in PLIN1 and a concomitant decrease in PLIN2 protein levels; therefore, these authors suggested that perilipins compete for the LD surface. In relation to this competition model, Ajjaji *et al.* compared the affinities of heterologously expressed PLIN1, PLIN2, and PLIN3 for LDs in Huh7 cells, concluding that PLIN1 displays the highest LD affinity, followed by PLIN2 and PLIN3.

In addition to transcriptional regulation, perilipin levels can be regulated by protein degradation. Besides previously mentioned studies implicating proteasomal degradation in the regulation of PLIN2, more recent work from the Cuervo group implicates CMA as an important regulator of adipocyte differentiation and adipogenesis [110]. This study shows that inactivation

of basal CMA, in mouse 3T3L1 cells and *in vivo*, leads to a blockage in adipocyte differentiation at an early commitment step, suggesting that removal of PLIN2 from the growing LD occurs *via* CMA and is required to progress to full differentiation and for PLIN2-to-PLIN1 exchange. Furthermore, *de novo* lipid synthesis (e.g., addition of FAs to cell media) and lipolysis, which induces PLIN1 phosphorylation and increases its stability, can also induce differences in perilipin targeting. In an engineered adipocyte-like cell system, PLIN2 was observed on LDs in response to lipolytic stimulation [111].

Finally, perilipin LD affinities and their distribution on LDs may vary according to LD lipid composition. Hsieh *et al.* [109] observed a striking difference between perilipins in their binding to sterol ester vs. TAG-enriched LDs when they are present in the same cell (in Y1 adrenal cells or in macrophages). However, a subsequent proteomics study on sterol ester or TAG-enriched rat granulosa stereogenic cells did not show a strong enrichment of perilipins on LDs under either condition [112]. Currently, little data are available regarding the variability in phospholipid composition of cellular LDs due to difficulties in obtaining sufficiently pure LDs for reliable analysis of their phospholipid composition. Recent discoveries of lipid transfer proteins that may mediate selective transport of phospholipids to or from LDs suggest that the LD phospholipid monolayer can be actively remodeled during the lifetime of an LD [113–116]. How LD lipid composition affects perilipin targeting remains to be explored.

Control of the lipolytic process by PLIN1

Lipid droplets are metabolic hubs controlling energy supply and lipid homeostasis in adipocytes. It is now well established that PLIN1 acts as a biological conductor of this metabolic hub, especially by regulating lipolytic processes. Lipolysis is the cellular process taking place on the LD to catabolize a TAG molecule into three FAs and one glycerol moiety. It proceeds in three steps: first, TAG is hydrolyzed to DAG by the adipose triglyceride lipase (ATGL); then, DAG is hydrolyzed into monoacylglycerol (MAG) by the hormone-sensitive lipase (HSL); and, finally, MAG is hydrolyzed into glycerol by monoacylglycerol lipase (MGL). HSL is highly expressed in adipose and breast tissue, as well as in the gastrointestinal tract, whereas ATGL and MGL are ubiquitously expressed. In WAT, the glycerol and FAs produced are liberated into the circulation to serve as substrates for oxidative

metabolism or gluconeogenesis, whereas, in oxidative tissues, they are taken up by the mitochondria for production of ATP [117]. Activation of ATGL requires a co-activator comparative gene identification-58 [CGI-58; also known as $\alpha\beta$ hydrolase domain containing 5 (ABHD5)]. It is also subject to inhibition by G0/G1 switch gene 2 (G0S2) and the recently identified hypoxia-inducible gene 2 (HIG2) [118].

PLIN1 was identified in an investigation of the phosphorylation and dephosphorylation processes of HSL under β -adrenergic stimulation as the most highly phosphorylated protein dependent upon cAMP-dependent protein kinase (PKA) [12]. It was soon recognized as the main regulator of lipolysis in adipocytes [119]. In agreement, *PLIN1* knockout mice show defects in lipolysis and resistance to obesity, although *PLIN2* expression is increased [120]. During the decades after its discovery, the role of phosphorylation of PLIN1 in adipocyte lipolysis has been investigated. Six sites of PKA-induced phosphorylation were identified in murine PLIN1: S81, S222, S276, S433, S492 and S517 [121], five of which are conserved in human PLIN1 (S81, S277, S436, S497, and S522; Fig. 4). However, a PLIN1 mutant with all 6 serines mutated to alanine still fully supported lipolysis [122]. This can be explained by more recent large-scale phosphor-proteomic studies, which have identified a number of additional phosphorylation sites in PLIN1, some of them also likely PKA-dependent [123]. The

phosphorylation of PLIN1 under β -adrenergic stimulation allows the translocation of HSL from the cytosol to the LD [124,125], even though a fraction of HSL might be LD-associated under basal conditions [122]. Because HSL itself is phosphorylated by PKA [126], the combination of these events leads to a large increase in its lipolytic activity. One or more serines in the N-ter region of PLIN1 were shown to be required for HSL translocation [89,127] (Fig. 4).

A direct interaction between HSL and PLIN1 was suggested by immunoprecipitation and Forster energy transfer (FRET) analyses in a heterologous expression system [89,128]. The N-ter region of PLIN1 was implicated in the interaction with HSL, although there was a discrepancy between the two studies in the exact mapping of the HSL interaction domain. Wang *et al.* [89] showed that the PAT domain was required for the interaction with HSL and suggested that this interaction and mechanism of HSL recruitment to LDs was shared with PLIN2, PLIN3, and PLIN5, but this result has not been confirmed by subsequent work. To date, the precise mode of HSL–PLIN1 interaction is not well understood.

In contrast to its positive role in the activation of HSL, PLIN1 acts as a repressor of lipolysis under basal conditions by sequestering the ATGL co-activator CGI-58/ABHD5 on the LD surface and preventing its degradation by the proteasome [72]. The C-ter region from aa 382 to 429 of PLIN1 is required for CGI-58

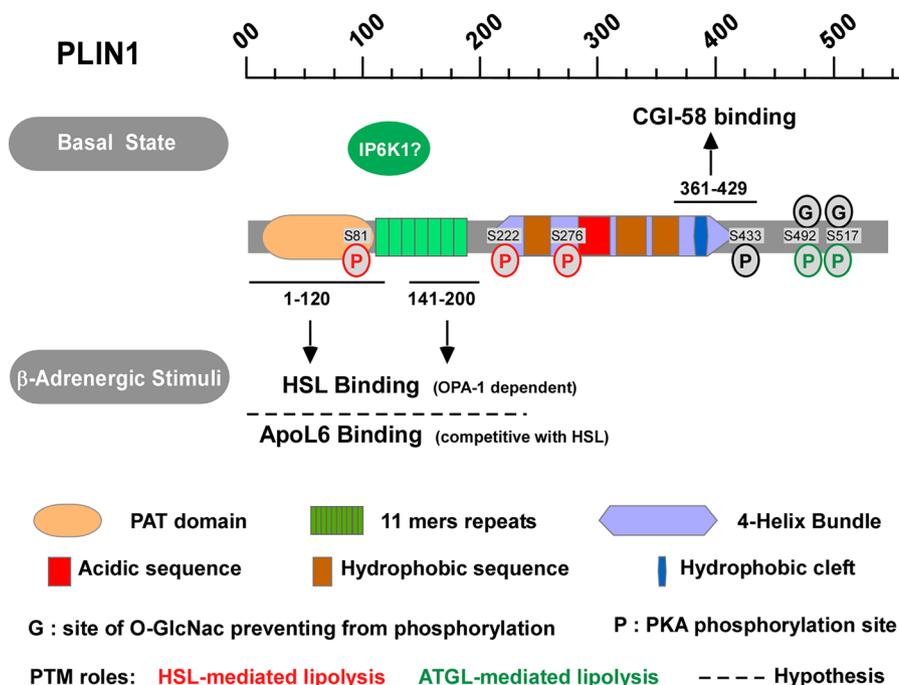


Fig. 4. PLIN1 function in lipolysis regulation. PLIN1 phosphorylation (P) and O-GlcNacetylation (G) sites and protein domains/regions implicated in the regulation of lipolysis and interaction with other proteins at basal state versus under β -adrenergic stimulation.

binding [72,129,130]. PLIN1 phosphorylation under β -adrenergic stimulation provokes the release of CGI-58, which then interacts with ATGL, activating the first step of hydrolysis [118,131]. Phosphorylation of the C-ter serine residues of PLIN1 (S492 and S517) is required for CGI-58 dissociation from PLIN1 and ATGL-dependent lipolysis [70,71] (Fig. 4). Small specific molecules blocking the PLIN1–CGI-58/ABHD5 interaction were identified in a chemical screen by the Granneman group [132], providing a useful tool to study the regulation of lipolysis by PLIN1.

Other types of post-translational modifications have been implicated in the regulation of PLIN1 activity: O-GlcNAcylation (O-linked β -D-N-acetylglucosamine adduct on serine or threonine residues) and sulfhydration (SH adduct on cysteine residues). An inducible adipocyte-specific knockout of O-GlcNAc transferase (OGT) led to elevated PLIN1 phosphorylation on S492 and S517, resulting in enhanced lipolysis in visceral fat and weight loss in mice [133], whereas OGT overexpression had the opposite effect and led to insulin resistance. Interestingly, the PLIN1 residues S492 and S517 were O-GlcNAcylated under basal conditions, suggesting that the balance between phosphorylation and glycosylation of PLIN1 may be important for the lipolytic response in adipose tissue. O-GlcNAcylation is implicated in the control of glucose homeostasis and is associated with insulin resistance and type 2 diabetes. Therefore, it may also play an important role in controlling lipolysis in human adipocytes [134]. PLIN1 sulfhydration by the cystathionine γ -lyase on cysteine residues, primarily present in the PAT domain, has been associated with reduced PLIN1 phosphorylation and thus reduced lipolysis by blocking HSL translocation to LDs [135,136]. More studies are needed to analyze the functional importance of this modification.

PLIN1 is highly regulated at the transcriptional level, as well as by protein degradation. *PLIN1* expression increases during adipocyte differentiation and is mainly dependent of PPAR γ transcriptional regulation [137] *via* a PPRE cis-element in its promoter [138]. On the other hand, PLIN1 can be degraded by either the proteasomal or the lysosomal pathway [29,79,139]. In adipose tissue of obese individuals, local pro-inflammatory cytokines impair TAG storage *via* autophagy induction. PLIN1 has been identified recently as the major protein triggered by TNF α -induced autophagy in the omental adipose tissue of obese individuals [140].

Besides HSL and CGI-58, several other PLIN1 partners that influence lipolysis have been identified. Two of them, optic atrophy 1 (OPA1) and apolipoprotein L6 (ApoL6), have been proposed to influence the interaction of PLIN1 with HSL. OPA1, an A-kinase

anchoring protein and a mitochondrial GTPase that organizes mitochondrial fission–fusion events, co-immunoprecipitates with PLIN1 and two regulatory PKA subunits, R α and RII β [141,142]. OPA1 is up-regulated during adipocyte differentiation and it relocates from mitochondria to LDs in mature human adipose stem cells. OPA1 was suggested to anchor PKA1 to LDs to promote PLIN1 phosphorylation at S497 and S522 upon β -adrenergic stimulation. In *OPA1*-silenced cells, HSL was retained in the cytoplasm even after isoproterenol treatment, suggesting that OPA1 regulates PKA-mediated phosphorylation of PLIN1. A very interesting recent preprint suggests that ApoL6 is able to inhibit lipolysis in 3 T3-L1 adipocytes by directly interacting with the N-ter region of PLIN1, thereby preventing its interaction with HSL [143]. Interestingly, the ApoL6–PLIN1 interaction appears to be independent of PLIN1 phosphorylation state. Mice with a global *ApoL6* knockout and fed a high-fat diet were leaner (with smaller WAT and adipocyte size) and displayed improved insulin and glucose tolerance. Absence of ApoL6 increases lipolysis *in vivo* to prevent diet-induced obesity. By contrast, mice with *ApoL6* overexpression displayed larger WAT and adipocyte size and were more glucose intolerant and insulin resistant under a high-fat diet. The proposed physiological mechanism relies on a decrease in ApoL6 levels during fasting, which allows PLIN1 to interact with HSL.

The role of other putative PLIN1 partners is less clear. *In vivo* knockout of inositol hexakisphosphate kinase-1 (*IP6K1*), which generates the signaling molecule inositol pyrophosphate, results in lean mice due to enhanced energy expenditure and basal lipolysis [144]. By mass spectrometry analysis of mouse WAT and cultured adipocytes, PLIN1 was identified as an interacting partner of IP6K1, and PKA/PKC-mediated phosphorylation of IP6K1, which is increased in mature adipocytes, was required for this interaction [145]. PLIN1 was also suggested to interact with aquaporins, especially aquaporin-7 (AQP7), plasma membrane channels that have been implicated in the release of lipolytically produced glycerol from adipocytes [146]. AQP7 was associated with PLIN1 LDs during lipogenesis, whereas the PLIN1–AQP7 interaction was highly attenuated during stimulated lipolysis and was sensitive to phosphorylation by PKA. Interestingly, immunofluorescence of primary human adipocytes shows PLIN1 in a patchy distribution on the unilocular LD upon insulin treatment, colocalizing with AQP7, whereas isoprenaline treatment results in PLIN1 dispersal [147,148]. Finally, a recent preprint using APEX2 proximity labeling identified about 70

proteins in the vicinity of PLIN1, including PLIN2, PLIN3, and PLIN4 [149]. Notable among the proximal proteins were members of the 14-3-3 protein family, scaffolding proteins known to bind to phosphorylated targets, protecting them from dephosphorylation. The PLIN1-14-3-3 interaction was lost under β -adrenergic stimulation and enhanced in the presence of insulin, suggesting a potential functional role in the anti-lipolytic action of insulin. However, the APEX2 proximity screen did not uncover any of the previous PLIN1 interacting partners, underscoring the difficulty of applying classical cellular and biochemical approaches to the atypical nature of LDs.

Function of other perilipins in lipolysis

PLIN1 is not detected in most cell types other than adipocytes, suggesting that other perilipins may be important for regulation of lipolysis. PLIN5 has been recognized as a key player for LD function in oxidative tissues such as the heart, skeletal muscle, liver, and brown adipose tissue (BAT), where it is highly expressed [20]. Granneman *et al.* [71] demonstrated a direct interaction between PLIN5 and ATGL in *PLIN5*-transfected 3T3-L1 cells. They later showed that PLIN5 interacts with CGI-58 and ATGL in a mutually exclusive manner [150]. Like PLIN1, PLIN5 is phosphorylated by PKA [27,151]. Pollak *et al.* [152] proposed that PLIN5 acts as a lipolytic barrier to prevent uncontrolled lipolysis by sequestering CGI-58 on LDs, thus preventing its interaction with ATGL. Phosphorylation by PKA may act as a switch between the lipolytic barrier and pro-lipolytic functions of PLIN5. Intracellular long-chain FA levels also influence PLIN5–CGI-58 interaction, as long-chain fatty acyl-CoA binding to CGI-58 has been shown to promote its interaction with PLIN5 [132]. PLIN5 has also been shown to associate with HSL on LDs in skeletal muscle [153], and in CHO cells in a constitutive manner [89]. How PLIN5 coordinates its interactions with ATGL, CGI-58, and HSL according to cellular needs remains to be investigated.

What role PLIN2, PLIN3, and PLIN4 play in lipolysis is not well understood. PLIN3 has the widest tissue distribution, followed by PLIN2. Neither of them has been shown to directly function in the activation of lipolysis by HSL or ATGL [30]. On the contrary, a global *Plin2* knockout mouse displays decreased TAG levels in the liver and shows resistance to diet-induced obesity, although the latter phenotype varies between different mouse models [29,154]. It was suggested that PLIN2 prevents the access of ATGL to the LD surface and thereby protects LDs against lipolysis [108,155].

In liver cells with a knockdown of *PLIN2* and *PLIN3*, LDs are highly enlarged and fewer in number, whereas ATGL-dependent lipolysis is increased [108]. These observations led the authors to suggest that PLIN2 and PLIN3 act as surfactants on the LD surface. In muscle cells, where PLIN2 is the most abundant perilipin, increased *PLIN2* expression likewise stabilizes LDs [82]. Loss of PLIN2 leads to a shift from glucose towards FA oxidation in cultured myotubes from a mouse knockout model [156]. Lipolysis can be induced by degradation of PLIN2 by CMA following its phosphorylation by AMPK or choline kinase (CHK) $\alpha 2$ [81,157]. In tumor cells, LD lipolysis is regulated to support cell survival and proliferation. It has been demonstrated that, upon glucose deprivation of brain tumor cells, CHK $\alpha 2$ binds to LDs and phosphorylates PLIN2 and PLIN3, driving their disassociation from the LD surface. Thus, CHK $\alpha 2$ promotes LD lipolysis, β -oxidation, and brain tumor growth [157]. Recently, PLIN2 has been implicated in the control of pluripotency of embryonic stem cells (ESCs). Wu *et al.* [158] have suggested that PLIN2 safeguards pluripotency of mouse ESCs by suppressing LD mobilization, whereas CMA leads to degradation of PLIN2, facilitating lipid hydrolysis and driving ESC exit from pluripotency. Altogether, these observations point to PLIN2, and possibly also PLIN3, acting as a shield to prevent lipolysis; the molecular basis for this shielding effect is not known.

The lipolytic function of PLIN4 has been explored even less than for the other perilipins. Although *PLIN4* expression is increased during adipocyte differentiation [75,77], a *Plin4* knockout mouse does not display a significant phenotype in terms of body weight or adipose tissue mass, with only slightly lower levels of cardiac TAGs and some reduction in *PLIN5* expression [159]. PLIN4 is also highly expressed in skeletal muscle, and, like PLIN2, PLIN3, and PLIN5, is more abundant in type I slow-twitch oxidative fibers, where it can be found enriched in the sub-sarcolemmal region, which is also where LDs are predominantly located [160]. Perilipins relocalize to LDs after lipid infusion in the muscle, with their levels and dynamics depending on exercise/training [161]. In cultured neurons from a mouse Parkinson's disease model, a reduction in *PLIN4* expression correlated with a decrease in LD abundance [162]. How these observations relate to the molecular function of PLIN4 is not known, but they could point to the importance of the long and repetitive AH of PLIN4, which can coat the neutral lipid core of LDs and decrease LD size in model systems [25,50], and could therefore also shield LDs from lipases.

Finally, it is interesting to consider the fruit fly *D. melanogaster* for possible clues about perilipin function in lipolysis. The *Drosophila* genome contains two perilipin genes, *Lsd-1/plin1* and *Lsd-2/plin2* (note that these do not correspond to mammalian PLIN1/PLIN2 but to a separate phylogenetic branch; see Fig. 2). *Lsd-1* mutant flies have large LDs and become obese, whereas *Lsd-2* mutant flies have small LDs, suggesting that the first protein stimulates and the second prevents lipolysis [163,164]. *Drosophila* also expresses two lipases, Brummer/dATGL and dHSL, as well as the ATGL co-activator CGI-58. LSD-1 is regulated by PKA-mediated phosphorylation and activates Brummer under stimulated conditions to promote lipolysis, whereas, in non-phosphorylated states, it prevents access of dHSL, demonstrating a high degree of functional homology with the mammalian system. Interestingly, *Lsd-1/Lsd-2* double knock-out flies displayed very low body fat levels when they hatched, but were able to recover as they aged to levels similar to *Lsd-2* mutants, and were also able to induce lipolysis upon starvation [164,165]. Beller *et al.* [164] concluded that perilipin-dependent control of cellular body fat in *Drosophila* is not essential for lipid metabolism, but may rather serve to fine-tune the system of lipid storage and mobilization to ensure efficient adjustments to actual energy requirements and food availability.

Nonlipolytic functions of perilipins

In addition to their regulation of lipolytic activity on the LD surface, perilipins have been proposed to interact with other LD-regulating proteins, for example CIDE proteins involved in LD–LD fusion, as well as proteins involved in LD–mitochondria contacts or in contacts between LDs and intracellular pathogens.

Lipid droplets form at discrete ER sites marked by seipin/BSCL2 and its partners, such as LDAF1. Seipin forms a large oligomeric complex in the ER bilayer that concentrates TAG and promotes LD nucleation [166]. As described above, PLIN3 can be observed on LDs within minutes after addition of oleic acid [45] and is used as a marker for nascent/early LDs [86]. A knockdown of *PLIN3* in HeLa cells reduced the number and size of LDs, leading Bulankina *et al.* [45] to suggest that PLIN3 was involved in LD formation; however, this observation can also be explained by a protective function of PLIN3 in lipolysis, as discussed in the previous section. Later studies by the same group showed a recruitment of Plin3 to ER membranes enriched in DAG, the precursor of TAG, upon treatment with a DGAT inhibitor or addition of DAG to permeabilized cells [48]. DAG was shown to

accumulate at the site of LD formation and was shown to promote their nucleation [167,168]. Recent studies confirmed the affinity of PLIN3 for DAG-enriched bilayer membranes [47]. Chung *et al.* [86] used endogenously tagged HALO–PLIN3 in SUM159 cells and observed colocalization between PLIN3 foci and both seipin and LDAF1, markers of nascent LDs. It was suggested that PLIN3 could promote LD formation by stabilizing DAG-enriched domains of the ER, but these results will require further investigation [49]. Recent data suggests that PLIN1 could also be involved in LD biogenesis. Jiao and collaborators worked on the identification of novel disease-gene interactions during differentiation in human adipocytes [169]. By means of multiple approaches, including immunoprecipitation and bimolecular fluorescence complementation in cultured human and mouse adipocytes, they found that PLIN1 interacts with seipin at ER–LD contact sites to promote lipid transfer to nascent LDs. Using atomic force microscopy, they also showed that a fraction of seipin dodecamers can bind directly to PLIN1 at ER–LD contact sites. Finally, using heterologous expression of *PLIN1*, *PLIN2*, or *PLIN3* in yeast incubated with ³H-palmitic acid, Jacquier *et al.* [15] observed accumulation of TAG or sterol ester, leading these authors to suggest that these perilipins promoted LD formation. Whether perilipins directly promote LD nucleation or budding requires further evaluation.

PLIN5 has the ability to tether LDs and mitochondria in different tissues. It contains a C-ter extension that is not present in other perilipins [27,170,171], with residues 425–463 with a conserved charged distribution being sufficient to induce LD–mitochondria contact sites [73,172]. Deletion of the final C-ter 3 aa of PLIN5 was sufficient to disrupt LD–mitochondria interaction, permitting Kien *et al.* [172] to study this function of PLIN5 without affecting its interactions with CGI-58 or ATGL, which also require the C-ter of PLIN5. Miner *et al.* [73] reported that PLIN5 interaction requires fatty acid transporter 4 (FATP4), a transmembrane protein involved in transport of long-chain FAs across membranes. However, the subcellular localization of FATP4 is currently not clear and FATP4 is more observed at the ER than at the outer mitochondrial membrane [173]. Another study showed that GTP-bound Rab8a was important in mediating PLIN5 interaction with mitochondria [174]. In both cases, the interaction with mitochondria depends on the extreme C-ter of PLIN5, and disrupting this interaction dramatically decreases LD–mitochondria contact sites and also impacts the transport of FAs from LDs to mitochondria in starved myoblasts [73], in L6

skeletal muscle cells, and *in vivo* [174]. Other proteins localized at the surface of mitochondria, such as mitofusin-2, may also interact with PLIN5 [73], but the physiological relevance of this interaction has yet to be established. In addition to PLIN5, a direct interaction between PLIN1 and mitofusin-2 was described in BAT to promote LD–mitochondria contact sites in response to adrenergic stimulation, and disruption of this interaction impaired lipolysis in BAT [175].

An increase in mitochondrial abundance surrounding LDs was observed in *PLIN2* knock-out myocytes [82], which could be compensated by an increase in PLIN5 levels, although this was not directly evaluated. The physiological relevance of the interaction between LDs and peridroplet mitochondria, which represent a distinct mitochondrial population [171], has been under intense investigation. A straightforward hypothesis is that the contacts direct FAs from LDs to mitochondria during lipolysis for β -oxidation or to protect cells from lipotoxicity [176,177]. This is supported by observations that LD–mitochondria contacts increase under conditions of energy stress, for example during starvation [178–180], or during exercise in muscle [181]. However, cold exposure of BAT reduced the association of mitochondria with LDs, whereas increased LD–mitochondria association significantly increased LD size and TAG levels, suggesting an opposite mechanism, whereby ATP produced by the mitochondria may promote FA–coenzyme A production, downstream TAG synthesis and LD growth. The LD–mitochondria association in BAT also at least partially depended on PLIN5 [171]. It is therefore possible that the regulation and function of PLIN5-mediated LD–mitochondrial contacts depend on the cell type and on specific metabolic conditions. A recent study in mouse AML12 hepatic cells or tissue suggests that peridroplet mitochondria facilitate FA esterification to prevent lipotoxicity and LD expansion, whereas cytoplasmic mitochondria support lipid catabolic pathways. A knockdown of *PLIN5* reduced the proteomic differences between the two mitochondrial populations, suggesting that PLIN5 regulates their function [182]. Close contacts between mitochondria, LDs, and the ER were observed in this study; however, PLIN5 did not impact the association between the ER and the mitochondria. Similar three-way organelle contacts have been observed in 3T3L1 adipocytes [113] and in other cell lines [114], with an implication of seipin and some lipid-transfer proteins. How these contacts affect perilipin distribution or function has not been further evaluated.

PLIN5 may also have a nuclear function by forming a complex with PGC-1 α /SIRT1, a transcriptional regulator involved in the regulation of reactive oxygen

species production/detoxification by promoting the expression of genes that mediate mitochondrial biogenesis and oxidative function [69,183]. Recently, PLIN5 has been identified as the FA-binding protein that specifically translocates mono-unsaturated FAs to the nucleus, leading to PGC-1 α /SIRT1 activation [69,183]. It is not known if this function is related to nuclear LDs (nLDs), which can be observed in the nuclei in some cell types, for example in hepatocytes after high-fat feeding, where nLDs are found close to nuclear membrane invaginations [184,185]. These nLDs are covered with PLIN3 and are largely devoid of PLIN2. Furthermore, deletion of PLIN2 does not affect nLDs, whereas it significantly decreases cytoplasmic LDs [185]. Sołtysik *et al.* [186] suggested that PLIN3 competes with the phosphatidylcholine synthesis enzyme CTP:phosphocholine cytidyltransferase α (CCT α) for the nLD surface and, by displacing it from the surface, induces downregulation of phosphatidylcholine synthesis.

Members of the CIDE protein family are important regulators of LD growth and fusion [187]. In mature white adipocytes, functional interaction between PLIN1 and FSP27 (CIDEc) has been shown to be required for efficient LD growth [188,189]. Co-expression of *PLIN1* with *CIDEc* specifically enhances CIDEc-mediated lipid transfer and LD fusion, whereas knockdown of *PLIN1* in adipocytes results in a significant decrease in LD fusion and growth [188]. The function of PLIN1 in controlling LD fusion and growth is dependent on its interaction with the N-ter region of CIDEc and is mediated by aa 292–319 in the acidic-residue-rich region of PLIN1. Recently, it was observed that the introduction of PLIN1 led to a noticeable reduction in the concentration of CIDEc proteins on the LD fusion plate and the authors of this study proposed that PLIN1 promoted formation of CIDEc condensates involved in neutral lipid transfer [190]. Further studies are required for a detailed understanding of this intriguing process.

Lipid droplets also accumulate in cells infected by pathogens in response to secreted virulence factors, providing microorganisms and viruses with substrates for effective growth. At the same time, these newly induced LDs can function as innate immune hubs that integrate cell metabolism and host defense and have been termed defense LDs (dLDs) [8,191]. Analysis of the dLD proteome revealed profound remodeling, particularly at the perilipin level, with decreased PLIN5 levels and less contact with mitochondria, and increased levels of PLIN2 and PLIN3 [8]. In contrast to their diminished contacts with mitochondria, dLDs engage in contacts with pathogens. It was suggested that PLIN2 could organize complex clusters of

defensive proteins on dLDs [8]. In dendritic cells, the action of interferon-inducible IGTP, which controls LD accumulation *via* action in phagosomal maturation, has been shown to depend on physical interaction with PLIN2 [192]. Control of lipolysis by perilipins is essential for proinflammatory molecule production, as described in adipocytes of *Plin1* knock-out mice that show sustained inflammation [193].

Molecular genetics of perilipins and human diseases

Members of the human perilipin family are encoded by five separate single-copy genes. *PLIN1* and *PLIN2* are located in the chromosomal region 15q26 and 9p22.1, respectively, whereas *PLIN3*, *PLIN4*, and *PLIN5* are co-localized in tandem in the 19p13.3 region. Analysis of sequence variations in the general population from the large genome sequencing aggregation database (gnomAD) reveals in the perilipin genes a common heterogeneity of single-nucleotide variations (SNVs) in terms of categories and allele frequencies [194]. Interestingly, the number of observed SNVs that predict a loss-of-function (pLoF) of perilipins is equal to or even greater than the number of theoretically expected pLoFs. In statistical terms, this observation means that the probability for perilipin genes to be intolerant to pLoFs (pLI) is close to zero. Conversely, genes with a high pLI score (> 0.9) are considered to be under high evolutionary pressure [195]. Thus, these epidemiological data on SNVs suggest that perilipin genes are rather tolerant to haploinsufficiency, which may seem counterintuitive, given the key role played by perilipins in the regulation of lipid storage and therefore in the maintenance of energy homeostasis. This could be explained by compensatory metabolic mechanisms that would limit the impact on survival and reproduction. As we will see below, these genomic epidemiology data are consistent with recent molecular genetic findings of perilipin-related pathologies. It is significant that the two Mendelian disorders linked to *PLIN1* and *PLIN4* dominant mutations do not result from haploinsufficiency but from a probable gain-of-function of mutant perilipin [196]. On the other hand, recent evidence shows that pLoF mutations of *PLIN1* and *PLIN4* influence, favorably or unfavorably, respectively, the distribution of body fat and metabolic health [197].

Mendelian disorders linked to perilipin genes

Only two Mendelian diseases, both with an extremely low incidence, have so far been linked to mutations in perilipin genes: a rare subtype of familial partial

lipodystrophy (FPLD4, MIM#613877) for *PLIN1*, and a vacuolar myopathy (MRUPAV, MIM#601846) for *PLIN4*. Both are autosomal dominant disorders resulting from heterozygous mutations responsible for aberrant protein synthesis, as explained below.

FPLD4 was first reported in three unrelated probands by the teams of Savage and Vigouroux, and was linked to frameshift mutations in *PLIN1* that lead to synthesis of a stably expressed elongated protein isoform with aberrant C-ter tail [198]. Like other subtypes of partial lipodystrophies, FPLD4 is characterized by the progressive loss of subcutaneous fat from certain regions of the body (limbs and trunk) and by metabolic complications resulting from ectopic fatty deposits, mainly in the muscles and the liver, which lead to insulin-resistant diabetes and severe hypertriglyceridemia (reviewed in [199]). Histological examination of subcutaneous adipose tissue from patients revealed a significant decrease in adipocyte size, macrophage infiltration, and increased fibrosis [198]. Since the first description by Gandotra *et al.*, seven other families of FPLD4 have been reported, with a total of five different FPLD4 mutations, three of which are recurrent [130,200,201] (Fig. 5).

Thus, the 398 fs mutation (c.1191_1192delAG) was detected in four different families and, for two of them, the haplotypic analysis led to the conclusion that they were due to two independent mutational events [198]. Functional analyses of *PLIN1* mutants, including 398, 404, and 439 fs, have shown decreased TAG accumulation and increased basal lipolysis in pre-adipocytes [199,200]. Since the domain between aa 380 and 427 of Plin1 has been shown to be crucial for binding to the ATGL co-activator CGI-58/ABHD5, it was hypothesized that mutants that lost this domain fail to sequester ABHD5, leading to a constitutive activation of lipolysis [202]. However, this hypothesis was not verified for the 439 fs mutant, which is able to bind ABHD5 [130]. These data suggest that the extended PLIN1 isoform may have special properties contributing to the pathophysiology of FPLD4. However, the nature of the deleterious role of the elongated variants remains elusive.

The involvement of *PLIN4* in the very rare MRUPAV autosomal dominant myopathy was recently demonstrated by Ruggieri *et al.* [203]. Using a multiomics approach, they showed in a large Italian family that an unusual expansion of a 99-nucleotide repeat sequence encoding the 33-mer unit of the PLIN4 repetitive AH domain [25] co-segregated with the myopathic trait. Maggi *et al.* [204] recently summarized the clinical characteristics of this slowly progressive adult-onset myopathy from 15 affected members of this

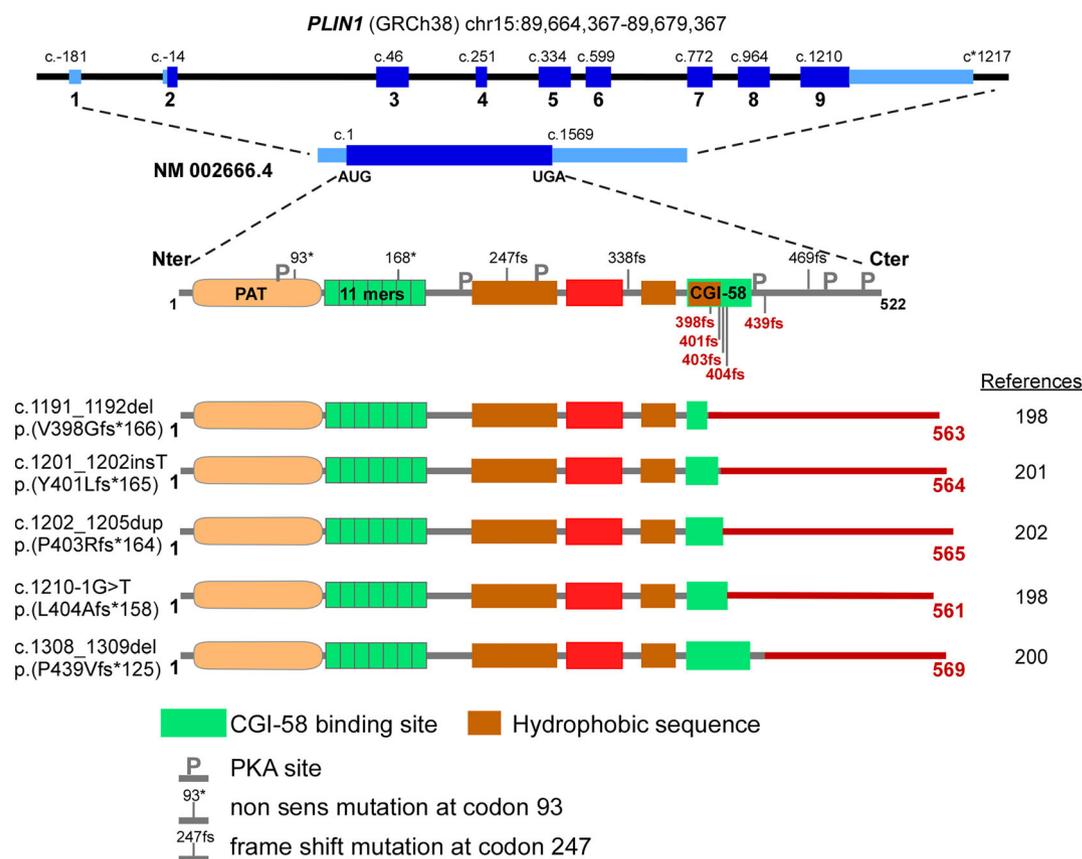


Fig. 5. Frameshift mutations in *PLIN1* associated with the familial partial lipodystrophy FPLD4. Variants indicated above the *PLIN1* diagram (e.g., 93*) are not associated with FPLD4, whereas frameshift variants indicated below the *PLIN1* diagram (e.g., 398 fs) are associated with the FPLD4 phenotype (red color).

exceptional four-generation family. Muscle biopsies of affected subjects showed the presence of rimmed vacuoles with subsarcolemmal aggregates, containing increased *PLIN4* levels as well as the ubiquitinated protein FK2 and autophagy markers p62/SQSTM1, which colocalized with *PLIN4*. Since there was also a significant positivity in the subsarcolemmae of aggregate-related protein NBR1, Ruggieri *et al.* [203] hypothesized that the expansion of the AH domain of *PLIN4* leads to its misfolding and, despite the activation of autophagy, *PLIN4* accumulation, which causes disorganization and dysfunction of the myofibers and their atrophy. Three other families and a sporadic case of MRUPAV have since been reported [205,206]. In two of these families, the *PLIN4* expansion appears identical in size to the one initially described, containing 39–40 instead of 29–31 repeat units. Notably, approximately 10% of carriers of the *PLIN4* expansion within the four MRUPAV families have been reported as asymptomatic, with one such carrier being over 50 years old [204]. Finally, in the third family, a

larger expansion consisting of 50 repeat units was identified in more severely affected subjects with an earlier onset myopathy (in their twenties), suggesting a correlation between the size of the expansion and the severity of the disease [205].

Common diseases and complex traits

The causes of common diseases such as obesity and cardiovascular diseases are multifactorial, resulting from the combined effects of environmental factors and genetic susceptibility factors. The latter can be very numerous but individually they play a weakly measurable effect in disease determinism. Genome-wide association studies (GWAS) involving thousands of single nucleotide polymorphisms (SNPs) make it possible, by identifying alleles in linkage disequilibrium among carriers of the trait, to find genetic susceptibility loci known as quantitative trait loci (QTLs). Hundreds of QTLs have thus been identified in common polygenic obesity and cardiovascular disease [207].

However, the validity of numerous GWAS is weakened by many biases, notably the fact that most analyzed SNPs are located in non-coding regions of the genome and their allelic frequency largely depends on the ethnicity of the studied populations [208]. On the other hand, candidate gene-association studies targeting genes such as the PLINs have been more fruitful, showing convincing associations between intragenic SNPs and complex traits. In recent years, the analysis of aggregated exome data from large cohorts of over 100 000 individuals with clinical and bioclinical information, such as the UK Biobank, has shown that rare coding variants are able to significantly influence quantitative traits such as biomarkers or anthropometric characteristics and thus to play a crucial role in the genetic determinism of polyfactorial diseases [209,210].

Common obesity

Due to their strong expression in adipose tissue and their key role in lipid storage regulation, early on, perilipins were the subject of research about the genetic determinants of obesity and its complications. The first *in vivo* findings come from *Plin1* knock-out mice, which are lean, have increased basal lipolysis and are resistant to diet-induced obesity but have glucose intolerance [120]. Conversely, overexpression of *PLIN1* in transgenic mice protects against diet-induced obesity and glucose intolerance [211]. In humans, clinical studies over the past two decades have shown that obesity is accompanied by significant variations in perilipin expression in adipose tissue [212]. In 2003, Mottagui-Tabar *et al.* [213] found a strong association between the intronic SNP rs894160 of *PLIN1* and decreased PLIN1A content in adipose tissue with increased basal lipolysis in a group of 117 obese Swedish women. Subsequent studies have provided compelling evidence on the relationship between some polymorphisms of *PLIN1*, such as rs894160 or rs1052700, and obesity (reviewed in [214]). Whereas several studies have confirmed the protective effect of the minor rs894160 allele against obesity in white women [215], others have shown non-significant results [216]. Divergent observations have also been reported regarding the resistance of obese subjects to weight loss based on their rs894160 genotype [217]. These apparent discrepancies between association studies, based on linkage disequilibrium with SNPs, reflect the complexity of interactions influenced by numerous factors, such as ethnicity, gender, nutritional intake, and lifestyle (dietary habits, physical activity, etc.), which likely modulate the results [218]. Another limitation arises from the fact that these SNPs are not directly responsible

for trait variation and that their link to the causal genetic determinant is statistically valid only for well-selected populations.

In the past decade, the availability of whole-exome sequencing (WES) data from thousands of individuals has paved the way for studies on the epidemiology of rare coding variants and provided powerful complementary approaches for new gene-related associations with complex traits. In 2022, several studies analyzing genotypic and phenotypic data from large populations have independently revealed an enrichment of predicted pLoF mutations in specific genes, including *PLIN1* and *PLIN4*, within subgroups of individuals phenotyped for obesity-related quantitative traits [197,207,219,220]. Koprulu *et al.* [197] have analyzed WES data from over 180 000 individuals from the UK Biobank and found that pLoF variants of *PLIN1* and *PLIN4*, as well as *PDE3B* and *ACVR1C*, were significantly linked with waist-to-hip ratio (WHR) adjusted for body mass index. Notably, pLoF variants in *PLIN1* were associated with a lower WHR and a more favorable metabolic profile, while *PLIN4* pLoF variants exhibited the opposite effect. This suggests that *PLIN1* haploinsufficiency may positively impact fat distribution and potential metabolic issues, whereas *PLIN4* haploinsufficiency could have a contrary effect.

Dyslipidemia and atherosclerotic cardiovascular diseases

For two decades, perilipins have been studied as candidate genes whose variations might contribute to disorders in lipid metabolism and atheromatosis. In 2004, Yan *et al.* [221] reported an association between *PLIN1* polymorphisms and an increased total cholesterol level in a population of obese and hypertensive Chinese people. Subsequently, other studies confirmed that certain alleles of common intronic *PLIN1* SNPs (rs894160 and rs10527000) negatively influenced the plasma lipid profile [218,222]. However, Perez-Martinez *et al.* [223] had shown in two populations (Spanish and American) an association between minor alleles of two *PLIN1* SNPs, including rs894160, and reduced post-prandial plasma TAG levels, potentially lowering the atherogenic risk.

In addition to *PLIN1*, studies suggest that variations in *PLIN2* and *PLIN5* might also influence the development of metabolic and cardiovascular diseases. Magné *et al.* [224] analyzing the S251P coding SNP of *PLIN2*, showed that the minor allele (P251) was associated with reduced plasma TAGs and hepatic very low-density lipoprotein (VLDL) secretion. After observing that *Plin5*^{-/-} mice had a worsened prognosis after myocardial ischemia, Drevinge *et al.* [225] investigated

the influence of a non-coding polymorphism (rs884164) of *PLIN5* in subjects with coronary artery disease. They found that the minor allele of this SNP was associated with unfavorable prognostic scores, suggesting that patients carrying this allele have a higher risk of cardiovascular morbidity and mortality after myocardial ischemia.

Recently, several large-scale population studies focusing on lipid metabolism biomarkers have revealed convincing associations between certain rare coding variations in *PLIN1* and *PLIN4* and plasma cholesterol and lipoprotein levels. In 2018, Klarin *et al.* [226] conducting a GWAS on blood lipids in 300 000 multi-ethnic individuals from the US Million Veteran Program, showed that carriers of the rare missense variant L90P in *PLIN1* exhibited significantly higher plasma HDL-C levels. This association was subsequently confirmed in the UK Biobank cohort [197]. Over the past 2 years, studies using UK Biobank data demonstrated a strong correlation between the presence of *PLIN1* pLoF variants and increased HDL-C levels coupled with reduced plasma TAG, suggesting that haploinsufficiency of *PLIN1* is associated with a favorable lipid profile [197,219,220]. These results were consistent with those from another large multi-ethnic cohort [210]. Moreover, individuals heterozygous for a *PLIN1* pLoF variant tended to have lower blood pressure, fewer diagnoses of hypertension, and fewer myocardial infarctions, indicating a lower overall risk of developing cardiovascular disease [197]. Interestingly, while over 40 pLoF variants scattered throughout *PLIN1* have been identified in a heterozygous state in several hundred individuals from the UK Biobank, none of the five frameshift variants responsible for the very rare FPLD4 have been detected. This confirms that these specific variants are not pathogenic due to haploinsufficiency, but rather through a mechanism that remains to be elucidated [196,197]. From the same UK Biobank GWAS, Koprulu *et al.* [197] found that *PLIN4* pLoF variants were associated with effects on blood lipids which, like the effects on body fat distribution, were opposite to those of *PLIN1* pLoF.

Insulin resistance and type 2 diabetes

PLIN1, through its control of lipase access to TAG stored in adipose tissue, may contribute in non-physiological situations to deregulation of lipolysis and excessive release of circulating FAs, leading progressively to insulin resistance and type 2 diabetes (T2D). Like GWAS on the adiposity trait, studies looking for associations between non-coding *PLIN1* SNPs such as rs894160 and the risk of T2D have produced

significant but sometimes discordant results depending on the ethnic origin of the populations studied [218,227]. Furthermore, the effect of *PLIN1* polymorphisms on T2D risk may differ depending on whether it is associated with obesity [227,228]. Obesity can deregulate not only lipolytic activity but also the endocrine function (adipokine secretion) of adipose tissue, indirectly contributing to T2D. Nutritional factors are also a source of bias that may explain the apparent discrepancy in some association studies. Thus, a strong correlation has been reported between *PLIN1* genotype and insulin resistance in subjects having a diet high in saturated fat and low in carbohydrates, irrespective of adiposity or ethnicity [229,230]. Interestingly, although some rare specific frameshift mutations in *PLIN1* are responsible for FPLD4, which includes severe insulin resistance [231], no association between many pLoF mutations and T2D has been reported to date, suggesting that haploinsufficiency of *PLIN1* is not a risk factor for insulin resistance [197].

PLIN2 plays a predominant role in lipid accumulation in the liver, the model of which is high-fat diet (HFD)-induced nonalcoholic fatty liver disease (NAFLD). NAFLD and insulin resistance are closely related conditions. *Plin2*^{-/-} mice subjected to HFD are resistant to obesity and hepatic steatosis and have adipose tissue that is more sensitive to insulin, with a greater tendency towards browning [232]. In humans, Sentinelli *et al.* [233] found in a large cohort of Italian obese subjects a strong association between the P251 variant of *PLIN2* and lower insulin secretion associated with increased insulin sensitivity. *PLIN5* also plays an important role in liver and muscle, and deletion of *PLIN5* in mice on HFD results in steatosis and insulin resistance in liver [234,235] and skeletal muscle [236]. However, to date, no association studies have demonstrated a relationship between *PLIN5* polymorphisms or coding variants and diabetes in humans.

Conclusion

The cumulated findings of the past two decades from numerous association studies demonstrate that perilipins contribute to the development of common obesity and atherosclerotic cardiovascular diseases, whose impact on public health is substantial. However, the mutational spectrum of the perilipin gene family appears to be variable and is indicative of the role of perilipins in the adaptive response of the genome to environmental constraints. Thus, there is on one hand a very small number of particular variants causing extremely rare Mendelian pathologies, the *PLIN1*-related FPLD4 and the *PLIN4*-related MRUPAV.

These *PLIN1* and *PLIN4* variants both generate specific protein isoforms whose pathophysiological mechanisms are still unclear. In contrast, numerous *PLIN1* and *PLIN4* pLoF variants have been reported in association with common pathologies linked to deregulation of lipid metabolism. However, counter-intuitively, the haploinsufficiency predicted by *PLIN1* pLoF variants appears to have a protective effect, reducing the risk of atherosclerotic cardiovascular disease in carriers. These observations are consistent with mouse models in which deletion of perilipin genes is associated with resistance to HFD-induced obesity. Collectively, these data suggest that the function of perilipins in promoting fat storage has not been strongly constrained during evolution, probably to allow for greater metabolic plasticity in response to random variations in natural macronutrient resources. Thus, perilipins might contribute to better survival when resources are low, but become pathogenic by promoting obesity and diabetes when resources are abundant. This suggests the existence of important epigenetic regulations that remain to be explored. Finally, the recent findings on perilipin gene-coding variants are very encouraging for the development of innovative therapeutic and preventive approaches to cardiovascular diseases and T2D related to metabolic syndrome.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Information concerning the sequences used to generate Fig. 2.