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## Lipin proteins and glycerolipid metabolism: roles at the ER membrane and beyond

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### Abstract

The regulation of glycerolipid biosynthesis is critical for homeostasis of cellular lipid stores and membranes. Here we review the role of lipin phosphatidic acid phosphatase enzymes in glycerolipid synthesis. Lipin proteins are unique among glycerolipid biosynthetic enzymes in their ability to transit among cellular membranes, rather than remain membrane tethered. We focus on the mechanisms that underlie lipin protein interactions with membranes and the versatile roles of lipins in several organelles, including the endoplasmic reticulum, mitochondria, endolysosomes, lipid droplets, and nucleus. We also review the corresponding physiological roles of lipins, which have been uncovered by the study of genetic lipin deficiencies. We propose that the growing body of knowledge concerning the biochemical and cellular activities of lipin proteins will be valuable for understanding the physiological functions of lipin proteins in health and disease.

### Keywords

phosphatidic acid phosphatase; triacylglycerol; rhabdomyolysis; lipodystrophy

## 1. Triacylglycerol and phospholipid biosynthesis

Recent ‘omics’ studies (such as LIPID MAPS and other large-scale lipid analysis projects) have revealed a vast range of cellular lipids, with more than 1000 species detected in a typical eukaryotic cell [1,2]. It has been estimated that approximately 5% of genes have a role in lipid synthesis [3], reflecting the critical role that lipids play in cellular structure and metabolism. The glycerolipids are abundant cellular lipids with physiological roles in energy storage (primarily in the form of triacylglycerol) and membrane structure (phospholipids and other lipids, varying by organelle type) [3]. Enzymes that regulate glycerolipid levels also play a role in modulating lipid intermediates that serve as signaling nodes to influence a wide range of physiological processes [4–6]. Both in their roles in signaling pathways and in energy storage and homeostasis, the enzymes that modulate glycerolipid levels have been of interest as potential drug targets [4,7].

Here, we briefly review glycerolipid biosynthetic pathways and then focus on the function of lipin phosphatidate phosphatase enzymes at the molecular and physiological levels. Of the enzymes in the glycerol 3-phosphate pathway, lipin enzymes are unique in their ability to transit among cellular membranes, rather than remain membrane tethered as most other enzymes in the pathway. This property of lipins allows them to act at diverse cellular organelles, and to have a role in processes beyond lipid biosynthesis. Some of these processes have been uncovered through the study of genetic lipin deficiencies, which will also be reviewed here.

### 1.1. The glycerol 3-phosphate pathway

As elucidated by Eugene Kennedy decades ago, glycerolipids are synthesized through modification of a glycerol 3-phosphate backbone [8,9]. Through acylation and dephosphorylation reactions, glycerol 3-phosphate is converted to triacylglycerol for neutral lipid storage, or is converted through other modifications to zwitterionic or anionic phospholipids (Figure 1). The regulation of reactions in the glycerol 3-phosphate pathway is one mechanism for controlling glycerolipid levels in the cell. The key enzymes driving the addition of fatty acids onto the glycerol backbone are glycerol acyltransferases (GPATs), acylglycerol-phosphate acyltransferases (AGPATs), and diacylglycerol acyltransferases (DGATs). In mammalian cells, each of these acyltransferase activities can be carried out by multiple proteins of the same class, including four GPAT enzymes and at least five AGPAT enzymes [10,11]. Some enzymes originally identified as AGPATs have subsequently been shown to have different enzyme activities.

In addition to the acyltransferase reactions, the *de novo* glycerophospholipid synthesis pathway employs a phosphatidate phosphatase (PAP) enzyme (Figure 1). In mammals, three independent genes encode the lipin 1, lipin 2 and lipin 3 proteins that catalyze this reaction [12]. Lipin protein activity catalyzes the conversion of phosphatidic acid (PA) to diacylglycerol [13,14], and thus acts at a branch-point for the synthesis of triacylglycerol, zwitterionic phospholipids (phosphatidylcholine and phosphatidylethanolamine), or anionic phospholipids (phosphatidylglycerol, phosphatidylserine, or cardiolipin) [15] (Figure 1). Lipin regulation of local levels of PA and diacylglycerol may also influence activation of signaling cascades that are regulated by these lipid intermediates [16]. The pathologies of lipin deficiencies appear to be associated with the loss of normal glycerolipid synthetic capabilities as well as aberrant regulation of PA-mediated signaling cascades, as discussed throughout this review.

### 1.2 Alternative enzyme activities in triacylglycerol synthesis

It should be noted that while most mammalian cells primarily utilize the glycerol 3-phosphate pathway, intestinal enterocytes possess an alternate pathway for triacylglycerol synthesis from monoacylglycerol, which is generated at high concentrations from the hydrolysis of dietary lipids in the intestinal lumen [17,18]. The monoacylglycerol pathway uses acylCoA:monoacylglycerol acyltransferase (MGAT) enzymes to generate diacylglycerol from monoacylglycerol. The resulting diacylglycerol is acted upon by DGAT enzymes. Therefore, TAG synthesis through the monoacylglycerol pathway does not require GPAT, AGPAT or lipin activities. It has been estimated that the monoacylglycerol pathway is

responsible for 70–80% of triacylglycerol synthesis for the production of chylomicrons in enterocytes [19,20]. However, these estimates are based on studies that were performed using chemical enzyme inhibitors prior to the isolation of genes for all of the enzymes shown in Figure 1. Recent studies in which MGAT2 or GPAT3 have been genetically ablated in the intestine suggest that the monoacylglycerol and glycerol phosphate pathways are non-redundant and each carries out a necessary role in intestinal lipid synthesis and metabolism [21,22]. Further studies are required to elucidate the relationship between the monoacylglycerol and glycerol phosphate pathways in intestinal lipid metabolism.

## 2. Activity and regulation of mammalian lipin proteins

The lipin genes were initially discovered by positional cloning in a spontaneous mutant mouse model known as fatty liver dystrophy (*fld*) [12]. The *fld* mice carry a null mutation in the gene now known as *Lpin1*, which prevents formation of any functional protein product. The *Lpin2* and *Lpin3* genes were identified based on their sequence similarity to *Lpin1* [12]. *Lpin1* mRNA undergoes alternative splicing to generate three isoforms: lipin 1 $\alpha$ , lipin 1 $\beta$  and lipin-1 $\gamma$  [23,24]. Lipin 1 $\alpha$  and lipin 1 $\beta$  exhibit different propensities for nuclear and cytoplasmic localization, although this may be cell-type dependent. Lipin 1 $\alpha$  is localized predominantly in the nucleus, whereas lipin 1 $\beta$  is primarily present in the cytoplasm [23]. Lipin 1 $\alpha$  and 1 $\beta$  isoforms are present in most of the tissues that express *Lpin1*, but the relative levels of the two isoforms varies with tissue type [23]. Lipin-1 $\gamma$  is present primarily in brain [24]. Lipin 1 $\alpha$  and 1 $\beta$  isoforms may have distinct cellular functions in processes such as preadipocyte differentiation. Lipin 1 $\alpha$  is associated with expression of adipogenic differentiation factors such as PPAR $\gamma$  and C/EBP $\alpha$ , and lipin 1 $\beta$  is associated with induction of lipogenic genes such as fatty acid synthase, stearoyl CoA desaturase, and DGAT [23]. Lipin 1 $\beta$  expression in adipose tissue of transgenic mice also leads to enhanced lipid accumulation in adipocytes, consistent with effects on lipogenesis [23,25]. It has been suggested that the ratio of  $\alpha$  and  $\beta$  isoforms of human *LPINI* is regulated by the splicing factor SFRS10 (Splicing factor, Arginine/Serine-Rich 10), with decreased levels of SFRS10 in obese subjects leading to greater propensity for production of the lipogenic lipin 1 $\beta$  isoform [26]. However, the association between SFRS10 levels and *LPINI* isoform mRNA ratio was not replicated in a subsequent study [27].

Attesting to the fundamental role of lipin protein activity in biology, orthologous lipin proteins are present in humans and other mammals, as well as plants, invertebrates and yeast [12]. The identification of lipins as PAP enzymes resulted from ground-breaking work by Carman and colleagues, who purified and sequenced yeast phosphatidic acid phosphohydrolase (Pah1) activity, and revealed it to be the same sequence previously identified as the yeast lipin ortholog [13]. Additional studies of yeast Pah1 by the Carman and Siniosoglou groups have provided detailed mechanistic insights into Pah1 regulation and degradation [28–37]. Many excellent reviews on Pah1 and yeast glycerolipid synthesis have been presented [38–41]. Here we focus primarily on the function of mammalian lipin proteins and the current understanding of their roles in physiology and pathophysiology.

## 2.1 Lipin Phosphatidic Acid Phosphatase Activity

All three mammalian lipin proteins have PAP enzyme activity *in vitro*, with specific activity for lipin 1 somewhat higher than that for lipin 2 and lipin 3 [14]. Lipin PAP activity requires  $Mg^{2+}$ , and is inhibited by N-ethylmaleimide [14,42]. Lipin enzymatic activity is specific for phosphatidate, with no detectable activity against a number of other lipid phosphates that have been tested [14].

The acyltransferase enzymes of the glycerol 3-phosphate pathway all possess a membrane-spanning domain and localize to the ER or mitochondrial membranes [11]. Unlike these proteins, lipin proteins do not possess a membrane spanning sequence, and lipin proteins may reside in the cytosol and associate with membranes in a transient manner. As discussed in subsequent sections, lipin proteins can also move to the nucleus and other cellular organelles. Prior to the molecular identification of lipins, PAP activity was known to relocate from the cytosol to the ER/microsomal membranes in the presence of fatty acids [43,44]; this translocation has been confirmed for lipin proteins [45,46].

It should be noted that another group of lipid phosphate phosphatase/transferase enzymes (known as LPPs or LPTs), which are structurally unrelated to lipin proteins, can also dephosphorylate phosphatidate [16,47]. LPPs differ from lipin PAP enzymes in their lack of requirement for  $Mg^{2+}$  and failure to be inhibited by N-ethylmaleimide [48]. By contrast to lipin PAP enzymes, LPP activity is not specific for PA and can utilize a number of lipid phosphate substrates (*e.g.*, lysophosphatidate, diacylglycerol pyrophosphate, sphingosine and ceramide phosphates) [49]. Furthermore, LPPs possess 6 transmembrane domains and reside at the outer surface of the plasma membrane, or the luminal surface of endomembrane compartments such as Golgi or ER membranes [50]. It is thought that a major portion of LPP activity may be carried out at the cell surface, where they hydrolyze extracellular lysophosphatidic acid or sphingosine-1-P and the resulting products activate intracellular signaling cascades in neighboring cells [16,50]. This ecto-enzymatic activity of LPPs may have a role in embryogenesis, tissue repair, development of atherosclerosis, and tumor growth and metastasis [51,52]. At intracellular membranes, LPPs have the potential to hydrolyze lipid substrates on the luminal side of ER or Golgi membranes, whereas lipin PAP activity acts at the cytosolic surface of these membranes. The intracellular lipid substrates for LPPs have not been clearly established. Consistent with distinct molecular roles of lipins and LPP enzymes, modulation of LPP or lipin levels in mouse models has distinct physiological effects [53].

## 2.2 Lipin transcriptional co-regulator activity

In addition to its role as a PAP enzyme in the glycerol 3-phosphate pathway, lipin proteins are implicated in the regulation of gene expression through interactions with nuclear receptors and other DNA-bound transcription factors [54]. The first demonstration of this was the requirement for lipin 1 in mouse hepatocytes for activation of genes regulated by peroxisome proliferator activated receptor  $\alpha$  (PPAR $\alpha$ ) and its coactivator protein, PPAR $\gamma$  coactivator-1 $\alpha$  (PCG-1 $\alpha$ ) by Finck and colleagues [55]. It is clear that lipin 1 also associates with additional transcription factors, including PPAR $\gamma$ , myocyte enhancer factor 2 (MEF2), and the nuclear factor of activated T-cells c4 (NFATc4) to co-activate, or in some cases, co-

repress, gene expression [56–58]. Lipin 1 mediated co-repression of MEF2 may modulate inflammatory cytokine signaling in adipocytes [57]. Lipin 2 also has the ability to co-activate PPAR $\alpha$ /PGC-1 $\alpha$  [46], but it is unclear under what physiological context this is important. The ability of lipin proteins to regulate both triacylglycerol synthesis through PAP activity, and fatty acid oxidation through transcriptional co-activation, suggests a unique mechanism for coordination of fat synthesis and turnover [55,59].

### 3. Regulation of lipin membrane localization

Cytosolic lipin proteins redistribute to multiple organelle membranes in response to stimuli such as fatty acids, or in the presence of negative charges such as conferred by the lipin substrate, PA (Figure 2) [15,43]. The dichotomous role for lipin proteins in glycerolipid biosynthesis and gene expression may be modulated, in part, by protein subcellular localization. Translocation of lipin to membranes should favor activation of lipin PAP activity for lipid synthesis, whereas localization to the nucleus may be necessary for its role as a transcriptional co-regulator. The association of lipin proteins with ER and nuclear membranes is conserved from mammals to invertebrates such as *C. elegans* and *Drosophila*. Similar to mammalian lipins, the *C. elegans* lipin ortholog is enriched in the ER and nuclear envelope membrane in cells of the embryo [60,61]. In *Drosophila*, a proportion of lipin localizes to ER and nuclear envelope membranes in the fat body and ventral ganglion cells [62]. This has provided impetus for identifying mechanisms that regulate lipin protein localization to membranes. Below we discuss some of the known determinants of lipin membrane association.

#### 3.1 PA-directed recruitment of lipin to membranes

Given that the ER is the primary site for synthesis of neutral lipids and membrane phospholipids, most studies of lipin membrane association have used ER membranes or artificial membranes. Collective evidence suggests that the biochemical basis of lipin protein– membrane association is the formation of a hydrogen bond between a polybasic domain in the lipin proteins and the PA phosphate group [63,64]. Lipin 1 and lipin 2 will physically associate with liposomes containing phosphatidylethanolamine and phosphatidylcholine in the absence of PA. However, the presence of PA greatly enhances the association efficiency [64]. It has been postulated that that cytosol-to-membrane redistribution of lipins may involve a two-step process. In the first step (known as the “bulk step”), soluble lipin enzyme associates with the membrane surface. This is followed by movement of the enzyme in the plane of the membrane to sites specifically containing PA substrate (known as the “surface step”) [64,65]. Little is known about how lipin proteins translocate to membrane surfaces in the “bulk step.” However, lipins (as well as other PA-binding proteins) may bind the PA substrate via hydrogen bonding between positively charged amino acids in lipins and the deprotonated form of PA [63,64,66]. Within the membrane, PA is an anionic lipid with a negatively charged phosphomonoester head group that can form hydrogen bonds with positive group donors, such as the primary amine group of phosphatidylethanolamine or other amphiphilic amines, in basic conditions. The formation of a hydrogen bond will deprotonate PA and create a di-anionic charged phosphate group that leads to increased electrostatic attraction.

It has been shown that a conserved polybasic motif in the N-terminal lipin domain is required for binding to PA-containing membranes [63]. In the three lipin paralogs, this motif is highly enriched in positively charged amino acids through a stretch of 9 lysine and arginine residues (amino acid residues 153–161 in mouse lipin 1). The hydrogen bond between lipin protein and PA serves both to enhance lipin–membrane association and to control PAP activity. Mutation of the polybasic motif in lipin 1 leads to reduced PA-mediated membrane association, consistent with lipin-membrane association occurring through an electrostatic interaction with negative charges in the membrane [63,64,67]. Conversely, the addition to liposomes of positive group donors such as PE or amphiphilic amines, and increasing the pH from 7 to 8, significantly enhances lipin 1 and lipin 2 membrane association and PAP activity [64,68]. Lipin 3 membrane interactions have not been experimentally tested, but conservation of the polybasic character of the corresponding region in lipin 3 suggests that similar interactions likely occur.

### 3.2 Fatty acid-induced lipin membrane translocation

As described above, fatty acids induce PAP enzyme translocation to the ER membrane. More specifically, unsaturated fatty acids, such as oleate, are potent inducers of lipin movement to the ER membrane, but saturated fatty acids are not [43–46,69,70]. It may be that unsaturated fatty acids increase membrane fluidity and PA movement, increasing the opportunity for interaction with the polybasic motif of lipin proteins [3]. Fatty acid-induced translocation of lipin is nearly completely inhibited by insulin, which would be released in a post-prandial state [45]. This suggests that the regulation of lipin 1 PAP activity via translocation to the ER membrane is complex and requires integrated signals provided by nutrients and hormones. There appear to be differences in the regulation of the lipin family members, as lipin 1 and lipin 2 are both translocated to the ER in response to oleate, but lipin 2 is not sensitive to insulin [46]. This suggests that additional factors contribute to the regulation of PA flux into neutral lipid storage and membrane phospholipid synthesis to meet cellular demand under different environmental circumstances. Fatty acid type may contribute to the regulation, through effects on the lipin-1 kinase -mTOR, for example. The saturated fatty acid palmitate activates the mTOR complex, whereas unsaturated fatty acids (including oleate and eicosapentaenoic acid) block the activation, which might influence lipin-1 phosphorylation and membrane association [71,72].

### 3.3 Lipin phosphorylation and membrane association

As initially demonstrated by Harris and colleagues, lipin 1 protein association with membranes is influenced by reversible protein phosphorylation [45]. mTOR mediates insulin-induced lipin 1 phosphorylation at multiple sites leading to cytosolic localization and reduced enzyme activity [45,64,68,73]. Inhibition of mTOR favors lipin 1 membrane association and increases its PAP activity [45,64,68,74]. Although studies suggest that lipin 1 phosphorylation might influence PA binding via the polybasic motif, neither of the two well-established mTOR-mediated phosphorylation sites on lipin 1 (Ser106 and Ser472) is located within the polybasic motif, suggesting that phosphorylation alters the protein conformation or its interaction with other proteins [64,74]. Many kinases that phosphorylate yeast Pah1 have been identified, but the phosphorylation sites in yeast and mammalian lipin proteins are not directly comparable, such that the sites, the kinases, and the physiological

consequences will require specific investigation in mammalian systems. Lipin 2 is also a highly phosphorylated protein, but unlike lipin 1, phosphorylation is not responsive to insulin or mTOR [68]. At present, little is known about the phosphorylation of lipin 3 and the effect of its subcellular translocation.

There is a complex relationship between mTOR, lipin 1 and PA: mTOR phosphorylates and regulates lipin 1, while the PA substrate of lipin 1 may itself regulate mTOR. Specifically, the PA generated from both the de novo synthesis pathway and hydrolysis by phospholipase D1 may bind to the FRB (FK506 binding protein-12-rapamycin binding) domain of mTOR protein to stabilize mTORC1 and mTORC2 complexes [75–77]. Interestingly, inhibition of de novo PA synthesis by AGPAT2 results in mTOR deactivation and lipin 1 subcellular redistribution in human pancreatic cancer cells [78]. On the other hand, exogenously supplied PA restores mTOR activation upon unsaturated fatty acid loading in two types of KRas-driven cancer cell lines [77]. These results suggest that PA dependence of mTOR activation might tune down lipin 1 PAP activity in rapidly proliferating cancer cells, which could promote PA flux into phospholipid synthesis for membrane biogenesis rather than neutral lipid storage. Thus, the interplay between mTOR and lipin 1 enzyme function appears to be more complex than simply kinase and phosphorylation substrate.

Lipin 1 protein phosphorylation is regulated by a phosphatase complex that was originally identified in yeast as Nem1 and Spo1 proteins, which were required to maintain formation of a spherical nucleus and for yeast sporulation [32]. The mammalian counterparts of these proteins are CTDNEP1 (formerly “dullard”) and NEP1-R1, which represent the catalytic and regulatory units, respectively, of the phosphatase complex [79,80]. The CTDNEP1/NEP1-R1 complex dephosphorylates lipin 1, promotes its association with the ER/nuclear envelope membrane, and stimulates its PAP activity in metazoan species from *C. elegans* to mammals [60,79,80]. A combination of three-dimensional structure analysis and *in vitro* steady-state kinetic analysis of CTDNEP1 showed that the phosphatase prefers to act at Ser106, one of the target sites for mTOR complex 1 phosphorylation [81]. However, to date there is no direct *in vivo* evidence that CTDNEP1/NEP1-R1 phosphatase activity counteracts the mTOR-mediated lipin 1 phosphorylation in mammalian cells. It remains unclear whether the dephosphorylation of lipin by CTDNEP1/NEP1-R1 affects the hydrogen bond formation between lipin and PA.

### 3.4 Lipin protein interactions

Lipin 1 can form homo-dimers, as well as heterodimers with lipin 2 or lipin 3 [82,83]. Despite the formation of oligomers, it appears that the individual lipin molecules present within oligomers act independently as phosphatases, rather than forming an active site through oligomerization [82]. The formation of lipin oligomers could influence lipin protein subcellular localization. This was demonstrated in a proof-of-principle experiment by fusing lipin 1 to a CAAX motif, which promotes membrane association through isoprenylation. This led to tethering of the tagged lipin 1 to membranes and the recruitment of other lipin molecules to the membrane site by interaction with the CAAX-tagged lipin 1 molecule [82]. These studies suggest that lipin proteins themselves may influence the localization of other lipin proteins within the cell.

Additional evidence for a role of lipin heterooligomers in protein localization comes from studies regarding the relationship between lipin 1 and lipin 3. Studies of mice with combined lipin 1 and lipin 3 deficiency revealed a cooperative role for lipin 1 and lipin 3 in adipose tissue development and triglyceride levels [83]. Investigation of the mechanism for lipin 1/lipin 3 cooperation in adipose tissue revealed that the combined presence of lipin 1 and lipin 3 influences their protein levels and subcellular localization of each. When expressed separately in HEK293 cells, lipin 1 and lipin 3 localized predominantly in the cytoplasm. When expressed in combination, a substantial amount of lipin 1 translocated from the cytoplasm to the peri-nuclear region, and lipin 3 appeared in the peri-nuclear region and in the nucleoplasm, in addition to the cytoplasm [83]. The co-expression of lipin 1 and lipin 3 from heterologous promoters that are not responsive to normal regulators of lipin gene expression led to increased lipin 1 protein and reduced lipin 3 protein levels [83]. This suggests that interaction of lipin 1 and lipin 3 influences their subcellular localization as well as protein concentration, the latter apparently occurring through a post-translational mechanism. Lipin 1 and lipin 2 also have been shown to reciprocally influence the levels of one another in cell systems and mouse tissues [84,85].

#### 4. Lipin association with diverse organelles

In addition to the ER membrane, lipin proteins may associate with the nucleus, lipid droplets, endolysosomes, and mitochondrial membranes [45,46,68,80,86,87]. Lipin localization to these diverse membranes is most easily conceptualized when one considers that many of these organelles form a network throughout the cytoplasm with a continuous lumen. For example, subdomains of the ER include the peripheral ER, the interconnected tubular network, and the nuclear envelope [88]. Furthermore, the ER has numerous membrane contact sites at the Golgi, mitochondria, peroxisomes, endosomes, lipid droplets, and the plasma membrane [41,88]. An important goal in the study of lipin biology is to understand the regulation of lipin subcellular localization to these distinct membrane and organelle sites, and the cellular and physiological role of lipins at each site. Below we discuss some of the current information regarding lipin protein association with organelles beyond the ER membrane and nucleus (Figure 2).

##### 4.1 Lipin 1 association with lipid droplets

As dynamic cytoplasmic organelles, lipid droplets (LDs) act as a reservoir for neutral lipids such as triacylglycerols and sterol esters, which serve as sources of energy and provide building blocks for membrane growth [89]. Lipid droplets are believed to initially form in specific regions of the ER where enzymes of the glycerol 3-phosphate pathway utilize fatty acids to synthesize triacylglycerol or sterol esters. LDs associate with specific proteins, such as members of the perilipin family. LDs may bud from the ER to form dispersed cytosolic LDs, or remain in contact with the ER to grow into large LDs through the association of glycerol 3-phosphate pathway enzymes such as GPAT4 and DGAT2, which migrate from the ER to LDs through ER-LD contact sites [90,91].

A comprehensive quantitative proteomics analysis in *Drosophila* S2 cells showed that at least one isoenzyme for each step of the triacylglycerol synthesis pathway (including



GPAT4, AGPAT3, DGAT2, and the *Drosophila* lipin ortholog) is associated with the LD fraction following oleate treatment [90]. In human macrophages, lipin 1 was shown to associate with the surface of LDs upon stimulation with a variety of compounds, including oleic acid, calcium ionophore, phorbol acids, and lipopolysaccharide [92]. A brain-specific lipin 1 splice variant, lipin 1 $\gamma$ , translocates to LDs in response to oleate loading when expressed in COS-7 cells [24]. The source of PA on LDs that could recruit lipin enzymes to the LD surface is not known. One possibility is that lipin associates with PA that is generated through the action of LD-associated AGPAT3.

Some evidence points to a potential role of lipin 1 in LD biology through an interaction with seipin, an ER protein that has a role in LD biogenesis. Seipin has been implicated in the process of ER surveillance to identify nascent LDs and promote their maturation [91]. Deficiency of seipin (encoded by the *BSCL2* gene) causes lipodystrophy in humans and mice [93–95], as does lipin 1 deficiency in the mouse [12,96,97]. Seipin knockdown leads to reduced lipin 1 binding to the ER membrane [98]. Physical interactions between seipin and lipin 1 proteins have been demonstrated in cell lines overexpressing these proteins, and this interaction was inducible by insulin [98]. Interestingly, seipin dodecamers were also shown to bind to AGPAT2 [99], which catalyzes the step preceding lipin action in the glycerol 3-phosphate pathway, and AGPAT2 deficiency also causes human lipodystrophy [100]. The lipin 1-seipin-AGPAT2 interactions are provocative and may provide a mechanism for channeling PA (the product of the AGPAT2 enzymatic reaction) to lipin 1 (which utilizes PA as a substrate). However, these findings require further investigation because the lipin 1-seipin-AGPAT2 interaction studies have all employed overexpression of epitope-tagged proteins in cell lines [98]. Verification of these interactions with endogenous proteins awaits the availability of specific antibodies against seipin and AGPAT2.

#### 4.2 Lipin 1 association with mitochondria

As described above, the PA-directed recruitment mechanism plays a central role in lipin membrane association. This mechanism may also underlie lipin 1 translocation to mitochondrial and plasma membranes. The mitochondrial-resident phospholipase D (MitoPLD) hydrolyzes cardiolipin in the membrane of mitochondria to generate PA (Figure 2). The overexpression of MitoPLD serves to recruit lipin 1 (specifically the lipin 1 $\beta$  splice variant) to the outer mitochondrial membrane [101]. Unexpectedly, a central domain of lipin 1 (amino acid residues 430 to 570), which contains only a few dispersed basic amino acids, was required for recruitment of lipin 1 $\beta$  to the mitochondrial membrane in response to MitoPLD overexpression [101]. In addition, the brain-specific splice variant, lipin 1 $\gamma$ , was shown to translocate from mitochondria/mitochondria-associated membranes to LDs in response to oleate loading [24]. The role of lipin proteins at the mitochondrial membrane merits additional attention, both at the molecular level and in terms of corresponding physiological effects.

#### 4.3 Lipin 1 association with endolysosomes/phagosomes

Lipin 1 activity at the endolysosomal membrane appears to play a role in autophagy by promoting the fusion of autophagosomes with lysosomes to form mature autolysosomes that are proficient for autophagic degradation [87]. As with the other membrane sites discussed

here, the surface of late endosomes/lysosomes is known to contain PA, likely generated by phospholipase D1 activity (Figure 2) [102,103]. Recent studies in mouse muscle and fibroblasts revealed that lipin 1 translocates to the surface of endolysosomes upon mTOR inhibition [87]. The resulting PAP activity at the endolysosomal membrane serves to produce diacylglycerol and activate a protein kinase cascade that leads to the fusion of lysosome and autophagosome membranes. The lack of lipin 1 prevents the fusion step in the assembly of mature autolysosomes and leads to defective autophagy [87], which may contribute to lipin 1-related myopathy that occurs in lipin 1-deficient humans and mice (see section 5).

#### 4.4 Lipin nuclear localization

Lipin localization to organelles such as lipid droplets, mitochondria, and endolysosomes appears to involve lipin interactions with PA on associated membranes. Additional factors influence lipin protein localization to the nucleus. As determined by studies of lipin 1, nuclear localization requires the same polybasic motif that is required for binding to PA-containing membranes [104]. Features in addition to the polybasic motif also appear to influence lipin 1 protein subcellular localization, since the lipin 1 $\alpha$  and lipin 1 $\beta$  splice variants, which both contain the polybasic motif, exhibit different propensities for nuclear and cytoplasmic localization [23]. Post-translational modification of lipin 1 has also been shown to influence nuclear localization. Sumoylation of two independent lysine residues on lipin 1 leads to nuclear localization in cultured neuronal cells [58]. It is unknown whether sumoylation regulates lipin 1 nuclear localization in additional cell types. In neuronal cells, mutation of the sumoylation sites prevents lipin 1 nuclear localization, raising the possibility that this modification may regulate lipin 1 activity in transcriptional co-regulation versus lipid biosynthesis. The sumoylation sites in lipin 1 are not conserved in lipin 2 or lipin 3, and it does not appear that these become sumoylated [58].

### 5. Lipin gene mutations and disease

As described in the preceding sections, a great deal of work has been done to define the biochemical and cellular functions of the lipin proteins. Another key area is the identification of the physiological functions of each lipin family member. All three mammalian family members have the same enzymatic activity and substrate specificity, albeit with somewhat different specific activities [14]. Lipin 1 and lipin 2 also exhibit coactivator/corepressor activity in cellular assays (lipin 3 is likely to exhibit similar activity based on sequence conservation) [46,55]. This raises questions about whether the three lipin proteins each have specific physiological roles, and if so, what they are. Valuable clues have been provided by the study of humans and mouse strains that carry rare lipin mutations, as well as mouse models with engineered mutations in lipin genes.

As described earlier, a spontaneous mutation in the mouse *Lpin1* gene produced a severe disease phenotype, indicating the physiological significance and non-redundancy of lipin 1 protein function. Rare mutations in human *LPIN1* or *LPIN2* genes have also been identified and found to cause severe, but substantially different, disease phenotypes. Individuals that carry bi-allelic pathogenic mutations in *LPIN1* present with severe, potentially life-

threatening rhabdomyolysis in childhood [105,106]. By contrast, individuals with bi-allelic pathogenic *LPIN2* mutations develop an autoinflammatory syndrome (Majeed syndrome) characterized by recurrent fever, bone lesions, anemia and inflammatory skin lesions [107,108]. The disparate symptoms associated with lipin-1 or lipin-2 dysfunction/deficiency indicate that these lipin proteins each have unique roles *in vivo*. At present, it is unknown whether *LPIN3* mutations cause disease. Large-scale exome sequencing in humans has identified numerous predicted pathogenic *LPIN3* mutations within the human population [109]. However, from these sequence data it is unclear whether there are individuals with pathogenic mutations in both *LPIN3* alleles, and if so, whether they are associated with disease.

Given the similar molecular activities of lipin 1 and lipin 2, what dictates the distinct disease pathologies? One contributor may be the tissue distribution of the two lipins. Recent high-throughput RNA sequence data for 50 human tissues from hundreds of individuals [110] allows the comparison of absolute expression levels of the three human lipin genes across tissues. As shown in Figure 3, mRNA counts reveal that *LPIN1* is the only lipin gene with significant expression in human skeletal muscle, which corresponds with the severe muscle pathology that characterizes human lipin 1 deficiency. As with humans, mice have very high *Lpin1* expression levels in muscle, as well as in adipose tissue and testes [5]. As in humans, *Lpin1* deficiency in the mouse is associated with muscle disease [87], but also with adipose tissue abnormalities and reproductive phenotypes, consistent with prominent expression in adipose tissue and testes [12,96,97]. Mouse and human lipin 1 are both expressed prominently in nerve tissue, and lipin-1-deficient mice exhibit abnormalities in peripheral nerve [111,112], but neurological symptoms have not been reported in lipin 1-deficient humans.

In contrast to *LPIN1*, the tissue expression pattern for *LPIN2* does not provide clear associations with the disease phenotype. *LPIN2* mRNA is most abundant in liver and intestine, tissues that are not known to be affected in Majeed syndrome. However, *LPIN2* is also expressed in blood (white blood cells) in humans (Figure 3) and in mice [46], which likely relates to involvement of lymphocytes and/or monocytes in the autoinflammatory symptoms of Majeed syndrome [113,114]. The lack of striking liver and intestinal phenotypes in Majeed syndrome may relate to the expression of *LPIN1* and/or *LPIN3* in those tissues to compensate for loss of lipin 2. In the following sections, we discuss some of the key roles of lipin proteins in specific tissues as revealed through the study of human disease and mouse disease models.

### 5.1 Lipin 1 in muscle and myocytes

The identification of *LPIN1* mutations in children with severe rhabdomyolysis was a revelatory discovery in lipin biology, which focused attention on a key role for lipin 1 in muscle [105,106,115]. Rhabdomyolysis is the destruction of skeletal muscle fibers with subsequent release of cellular contents, including high concentrations of myoglobin. In addition to the primary damage to muscle, the necessity to clear high levels of myoglobin from the circulation can lead to kidney failure. *LPIN1*-related rhabdomyolysis is a recessive condition, requiring pathogenic mutations in both alleles of the *LPIN1* gene. Analysis of 21

individuals with childhood rhabdomyolysis revealed bi-allelic *LPIN1* mutations in 17 (59%), suggesting that mutations in the *LPIN1* gene may account for a substantial proportion of childhood metabolic rhabdomyolysis cases [106]. In affected individuals, there is typically no evidence of the disease until a severe episode of muscle pain, often occurring at 2–5 years of age [105,116]. It is not fully understood how episodes of rhabdomyolysis are triggered, but they may be precipitated by fever, fasting, or anesthesia [105,116]. Sadly, up to one-third of affected individuals die during a childhood bout [106,115]. Fortunately, in recent years, the clinical awareness of *LPIN1* mutation and disease course has grown, leading to better management of cases when they appear in hospital emergency rooms [117].

The majority of pathogenic *LPIN1* mutations are null alleles that produce no protein product, resulting from nonsense, frame-shift, or deletion mutations [105,106,118,119]. Consistent with this, primary myoblasts from patients exhibit dramatically decreased PAPI activity [120]. A few pathogenic missense *LPIN1* alleles have been identified, and have been valuable to dissect the functional deficiency that underlies the observed muscle symptoms. For example, lipin 1 protein bearing the pathogenic missense mutation, Arg725His, retains co-activator function but lacks PAP activity, confirming that the pathology of lipin-1 rhabdomyolysis is a result of PAP deficiency [119]. Another missense mutation (Glu769Gly) was associated with severe myopathy in a heterozygous carrier when treated with statin drugs [105]. This raises the interesting possibility that *LPIN1* mutation may be a risk factor for statin-related myopathy, which occurs in a proportion of individuals taking statins to combat hypercholesterolemia [121].

How does lipin 1 deficiency cause rhabdomyolysis? As mentioned earlier, lipin 1 is the only lipin family member with appreciable expression in skeletal muscle of humans and mice. This may explain why lipin 1 deficiency is most profoundly experienced in skeletal muscle, where other lipin family members are not available to compensate. Involvement of cardiac muscle has been noted in a minority of lipin 1-deficient patients, but this is not a typical finding [106,115]. Histological analysis of muscle biopsies isolated from lipin 1-deficient patients, as well as lipin 1-deficient mice, revealed an unexpected accumulation of neutral lipid droplets [87,120,122]. The neutral lipids accumulating in lipin 1-deficient muscle were determined in mice to be cholesteryl esters [87]. Cholesteryl ester accumulation likely occurs in response to impaired triacylglycerol synthesis in the absence of lipin 1, as a compensatory mechanism designed to minimize the accumulation of free fatty acids, which would normally be incorporated into triacylglycerols.

Further analysis of lipid content by quantitative mass spectrometry was performed in two human patients with *LPIN1* mutations and in lipin 1-deficient mice [87,105]. The changes observed in phospholipid content is surprisingly mild, without significant changes in major membrane phospholipid classes, although some alterations in minor phospholipid species were observed. One of the human patients analyzed had elevated lyso-phospholipids, but the other did not [105]. In lipin 1-deficient mice, total phospholipids were not elevated, but the levels of ceramides and ether-phosphatidylcholine were increased, which may represent an adaptive mechanism for free fatty acid esterification in the absence of triacylglycerol formation [87]. Importantly, a common finding in mouse and human lipin 1-deficient muscle is an accumulation of PA [87,105], which may influence signaling pathways and processes

such as autophagy (discussed below). Studies with primary myoblasts from lipin 1-deficient humans have revealed a possible interplay between altered lipid metabolism and inflammation as a contributing factor to lipin 1-associated rhabdomyolysis. Abnormalities in gene expression in human lipin 1-deficient myoblasts were observed when treated with pro-inflammatory cytokines (IL-1 $\beta$  and TNF $\alpha$ ) that may influence fatty acid synthesis/oxidation [120].

The *fld* mouse model with lipin 1 deficiency provides a valuable model to elucidate the functions of lipin 1 in muscle in normo- and pathophysiology. The original studies of the *fld* mouse noted effects of the mutation in liver, nerve and adipose tissue [96,97,111]. No muscle defects were in evidence. Several years later, the identification of lipin 1-deficient humans provided an explanation: the clinical symptoms associated with lipin 1 deficiency (muscle pain, elevated creatine kinase levels due to muscle breakdown) are episodic, triggered by metabolic stress [105,116]. Furthermore, unlike humans, mice cannot easily communicate to their handlers that they are experiencing muscle pain, and so it was not evident that they are susceptible to muscle symptoms.

To make use of the *fld* model to study the basis for rhabdomyolysis in lipin 1-deficiency, it was necessary to identify conditions that could provoke 'episodes' of impaired muscle metabolism. Subjecting *fld* mice to a regimen of fasting for 16 hr followed by refeeding for 4 hr caused elevations in circulating creatine kinase levels, signifying muscle damage [87]. These conditions were used to characterize the effects of lipin 1 deficiency on muscle pathology, lipid profile, and mitochondrial function. It became clear that with the addition of fasting/refeeding as a metabolic stressor, the lipin 1-deficient mouse model recapitulates the changes in muscle lipid content observed in human muscle, including accumulation of PA and cholesteryl esters [87]. Muscle from *fld* mice also exhibited evidence of myocyte turnover, with > 10% of myocytes representing newly differentiated cells, presumably replacing dead myocytes. Lipin 1 deficient mice also have impaired muscle regeneration in response to injury [123], which could contribute to the severe muscle pathology in mice and humans lacking lipin 1.

In addition to muscle histopathological changes, lipin 1-deficient muscle has altered energy metabolism, including reduced mitochondrial respiratory reserve capacity [87]. Electron microscopy revealed an accumulation of aberrant mitochondria and presence of autophagosome structures. The latter are intermediates in the process of macroautophagy, and are typically short-lived. Their detection in the *fld* muscle, together with accumulation of potentially damaged mitochondria, suggested a defect in autophagic clearance. Interestingly, *Drosophila melanogaster* made deficient for the single lipin ortholog in the fruitfly fat body exhibit similar structural abnormalities of mitochondria and autophagosomes [124]. These observations led to the identification of a role for mammalian lipin 1 in autophagosome maturation. Lipin 1 conversion of PA to DAG on the surface of lysosomes activates a protein kinase cascade that leads to production of phosphatidylinositol 3-phosphate and fusion with autophagosomes to mature autolysosomes [87]. The impaired formation of autolysosomes in lipin 1-deficient muscle may contribute to dysfunction through impaired turnover of damaged organelles, particularly mitochondria, and subsequent changes in lipid and energy homeostasis. It is likely that lipin 1 deficiency also affects

function of additional organelles in muscle, potentially through changes in membrane lipid composition, to bring about the severe consequences of lipin 1 deficiency. This merits further investigation.

## 5.2 Lipins in adipocytes

A hallmark feature of adipose tissue is the storage of triglyceride droplets, which can be lipolyzed during fasting and other conditions to supply fatty acids locally and to distant tissues. Consistent with an important role for lipin 1 and the glycerol 3-phosphate pathway in adipocyte triglyceride synthesis, the *fld* mouse exhibits generalized lipodystrophy, with 90% reduction in the mass of white adipose tissue depots (including epididymal/ periovarian fat, inguinal fat, retroperitoneal fat, and omental fat) [97]. Lipid profiles show a dramatic reduction in triacylglycerol levels, with an accumulation of PA [125]. The cells isolated from the primordial fat depots in *fld* mice exhibit dramatically reduced expression of PPAR $\gamma$  and other adipogenic transcription factors that are required for differentiation [126]. Similarly, stromal vascular preadipocytes and mouse embryonic fibroblasts isolated from *fld* mice fail to induce an adipogenic transcription program that occurs as the first steps in differentiation [125,126]. These observations point to a role for lipin 1 in the early stages of adipocyte differentiation that precede triglyceride synthesis and storage. One contributor appears to be the elevated levels of PA that accumulate in *fld* pre-adipocytes, which activate ERK signaling and inhibit adipocyte differentiation [125]. Differentiation of *fld* preadipocytes can be rescued by providing constitutively expressed PPAR $\gamma$  or by ERK inhibitor [125,126]. It can also be rescued by forced expression of lipin 1, lipin 2, or lipin 3, but not by introduction of lipin 1 protein that possesses coactivator function without PAP enzyme activity [125]. These results strongly suggest that lipin PAP activity is required for the early stages of adipocyte differentiation, potentially as a regulator of PA signaling events.

Beyond the *fld* mouse model, an engineered *Lpin1* allele that leads to lipin 1 deficiency specifically in adipose tissue confirms the requirement for lipin 1 PAP activity for normal adipose tissue development *in vivo*. Finck and colleagues generated a “separation-of-function” mutant *Lpin1* allele that encodes a lipin 1 protein lacking PAP activity but retaining coactivator function [127]. The resulting mice exhibit lipodystrophy similar to that in the *fld* mouse (including reduced glyceride content and increased PA levels in adipose tissue) indicating that this phenotype is associated with loss of lipin 1 PAP activity. Additionally, these studies revealed an unexpected role for lipin 1 in regulating adipose tissue triglyceride lipolysis. This occurs through lipin 1 modulation of PA levels to influence a phosphodiesterase 4-cAMP-protein kinase A signaling axis to control lipolysis [127].

In contrast to effects of lipin 1 deficiency, increased lipin 1 expression levels in the mouse via an adipocyte-directed transgene lead to obesity, with increased size and triglyceride content in adipocytes [23,25]. Interestingly, the obese adipose-specific lipin 1 transgenic mice have improved glucose and insulin homeostasis compared to their wild-type counterparts on both chow and high fat diets [23]. These findings suggest that increased capacity for triglyceride synthesis within adipocytes via lipin 1 is metabolically favorable, perhaps by partitioning fatty acids into adipose tissue and away from ectopic storage in other tissues. In humans, lipin 1 expression levels in adipose tissue are positively correlated with

insulin sensitivity in both lean young adults and in obese subjects [128,129]. Lipin 1 expression levels increase both *in vivo* and *in vitro* in response to thiazolidinedione drugs, which are PPAR $\gamma$  agonists [129,130]. In cultured adipocytes, lipin 1 was required for PPAR $\gamma$ /thiazolidinedione stimulated adipocyte differentiation [130]. The induction of lipin 1 by thiazolidinedione drugs may be a mechanism for increased adipocyte differentiation observed with activation of PPAR $\gamma$  [130], as well as a mediator of the improved insulin sensitivity that occurs on a background of increased adiposity with these drugs [131].

Lipin 2 and lipin 3 also influence lipid metabolism in mouse adipocytes. In the mouse 3T3-L1 adipocyte cell line, lipin 1 and lipin 2 are reciprocally regulated during adipocyte differentiation [85]. Lipin 2 protein is detectable in preadipocytes and diminishes in mature adipocytes, while lipin 1 protein is present primarily after initiation of differentiation and continuing to highest levels in mature adipocytes [85]. Knockdown of both lipin 1 and lipin 2 after the induction of adipocyte differentiation leads to increased PA levels, but does not alter total triglyceride content of the mature adipocytes. Instead, lack of lipin 1 and lipin 2 in mature 3T3-L1 adipocytes leads to fragmented lipid droplets of reduced size and increased number [85]. The fragmented lipid droplet phenotype can be rescued by introduction of lipin 1 that possesses PAP activity, but not by lipin 1 with coactivator function that lacks PAP activity. Interestingly, lipin 2-deficient mice do not exhibit lipodystrophy or an obvious adipose tissue phenotype [84], suggesting that *in vivo* compensation by lipin 1 is adequate for normal adipose tissue. Mouse lipin 3 is also induced during differentiation of 3T3-L1 cells, and has a role in adipose tissue PAP activity and lipid accumulation in combination with lipin 1 [83]. Stromal vascular cells isolated from lipin 3-deficient mice exhibit impaired differentiation *in vitro*, but lipin 3 deficiency *in vivo* does not cause lipodystrophy, likely due to compensation by lipin 1. Consistent with this, the loss of lipin 1 and lipin 3 in combination cause even more severe lipodystrophy than lipin 1 deficiency [83]. Thus, the three mammalian lipins each have a demonstrable effect on lipid metabolism in mouse adipocytes, but lipin 1 appears to be the dominant PAP enzyme for triglyceride accumulation in mouse adipose tissue *in vivo*.

As summarized above, a role for lipin 1 in mouse adipose tissue development and lipid accumulation is clear. It was therefore unexpected when human lipin 1-deficient subjects were found not to have lipodystrophy [105]. *LPIN1*, *LPIN2* and *LPIN3* are each expressed to some degree in human primary adipocytes [83] and in the human SGBS cell line (isolated from an individual with Simpson–Golabi–Behmel syndrome) [132]. *LPIN1* knockdown in SGBS cells led to a 95% reduction in PAP activity, but TAG accumulated to the same level as wild-type cells [132]. *LPIN2* or *LPIN3* knockdown led to more modest (10–25%) reductions in PAP activity, with maintenance of normal (*LPIN2*) or slightly reduced (*LPIN3*) TAG levels. Surprisingly, combinatorial silencing of all three human lipins led to only a 40% reduction in cellular TAG accumulation, indicating that after dramatic reduction in cellular lipin content, alternative mechanisms act to promote TAG accumulation. However, it should be noted that the knockdown of the lipin proteins in SGBS cells was not complete; triple knockdown cells still retained lipin protein levels ranging from about 5% (for *LPIN1*) to 50% (for *LPIN2*) of levels in wild-type cells. Two additional mechanisms may compensate for reduced lipin levels as well. These are an approximately 50% reduction in lipolysis of stored TAG, as determined by glycerol release, and increased lipid phosphate phosphatase

(LPP) activity in SGBS cells having triple lipin knockdown [132]. It will be interesting to determine whether similar compensatory mechanisms occur in adipose tissue of lipin 1-deficient humans to protect them from lipodystrophy, but cannot protect against other pathologies that occur in lipin 1-deficient muscle, such as altered energy metabolism and myocyte differentiation.

### 5.3 Lipins and inflammation

As described in an earlier section, lipin 2 deficiency in humans leads to an autoinflammatory disease known as Majeed syndrome, characterized by episodic sterile bone lesions, anemia, and skin inflammation in some patients. Majeed syndrome patients have elevated levels of proinflammatory cytokines. The *LPIN2* mutations in Majeed syndrome include frame-shift mutations that lead to premature stop codons and a missense mutation, Ser734Leu [107,108,133]. Lipin 2 has both PAP activity and transcriptional coactivator activity [14,46], but it was unknown whether loss of one or both of these functions causes Majeed syndrome. Analysis of Ser734Leu lipin 2 revealed that this mutant protein has lost the ability to catalyze the PAP reaction, but retains transcriptional coactivator activity, indicating that lipin 2 PAP activity is the critical component in Majeed syndrome [46]. Interestingly, five other lipin 2 missense mutations are associated with psoriasis (Infevers database, <https://fmf.igh.cnrs.fr/ISSAID/infevers>).

A lipin 2-deficient mouse model was generated to characterize the physiological roles of lipin 2 [84]. These mice exhibited dyserythropoietic anemia similar to that reported in Majeed patients, as well as occasional skin lesions. This mouse model also revealed that loss of lipin 2 activity in liver is compensated by upregulation of lipin 1, and that lipin 2 is critical for maintenance of lipid homeostasis in the cerebellum as mice age. Lipin 2-deficient mice also had reduced trabecular bone density. However, analysis of tibiae via radiography and micro-computed tomography failed to reveal evidence of inflammatory bone lesions that are a hallmark of Majeed syndrome. It is possible that more sensitive techniques are required to observe bone lesions, such as isotope uptake techniques that are used in human diagnoses, or that the episodic nature of the appearance of Majeed symptoms prevented observation of the same effects in the mouse. Better understanding of triggers for severe episodes of bone inflammation may allow induction of similar episodes in the mouse model, which would be valuable for elucidating the molecular mechanisms.

Fortunately, recent studies have begun to elucidate the inflammatory dysregulation that occurs in Majeed syndrome. The treatment of Majeed syndrome patients with anti-inflammatory drugs provided an important clue to the inflammatory mechanism involved. Anti-inflammatory drugs that target tumor necrosis factor  $\alpha$  had no effect on Majeed syndrome symptoms, but blockade of interleukin 1 $\beta$  (IL-1 $\beta$ ) led to improvement, suggesting that elevated IL-1 $\beta$  levels play a role in the disease pathogenesis [133]. Indeed, a recent study from Balboa and colleagues has shed light on the relationship between lipin 2 and IL-1 $\beta$  [114]. Lipin 2 knockdown in human macrophages, or lipin 2 deficiency in mouse bone marrow-derived macrophages, led to enhanced IL-1 $\beta$  production in response to activation of the NLRP3 inflammasome, a multiprotein complex that is normally activated by exposure to pathogens and other danger signals, and serves to initiate the innate immune



response [134]. This led to the identification of several mechanisms by which lipin 2 influences inflammasome activation. The NLRP3 inflammasome requires two independent signals for activation. The first is a priming signal that occurs via activation of cellular receptors such as the toll like receptors, and leads to IL1 $\beta$  transcription. The second signal may consist of pathogens or cellular stress signals such as ATP, which signal through receptors such as the purinergic receptor, P2X<sub>7</sub> [134]. Lipin 2 appears to influence multiple steps in inflammasome activation, including regulation of MAPK signaling to regulate IL-1 $\beta$  production during inflammasome priming, P2X<sub>7</sub> sensitization, and processing of the caspase to its active form that ultimately leads to processing of IL-1 $\beta$  from its precursor to mature form [114]. IL-1 $\beta$  overproduction in response to lipin 2 deficiency could be prevented by MAPK inhibitors or by restoration of reduced cholesterol levels in lipin 2-deficient cells to wild-type levels. These studies reveal a novel nexus between lipin 2 and cholesterol metabolism and the innate immune response. The further understanding of this relationship may provide new insights into innate immunity, and provide novel treatment strategies for autoinflammatory diseases.

## 6. Summary and Future Perspectives

Studies of the multiple lipin family members in human disease and mouse models have led to unifying themes regarding the cellular function of lipin proteins, which in turn, impact their physiological roles. Themes that have arisen from the study of lipin biology include: (1) the ability of lipin proteins to traffic to multiple cellular locations, including membranes of different organelles; (2) the modulation by lipin PAP activity of the concentration of bioactive signaling lipids phosphatidic acid and diacylglycerol at specific membrane sites; (3) a need for lipin proteins in tissues that are not traditionally major sites of triacylglycerol synthesis and storage, such as skeletal muscle and macrophages; and (4) the three mammalian lipin family members each have unique physiological roles, but that they may cooperate with, and/or compensate for, one another. Future studies should take advantage of the growing body of knowledge concerning the biochemical and cellular activities of lipin proteins to inform investigations of lipin physiological function in health and disease.

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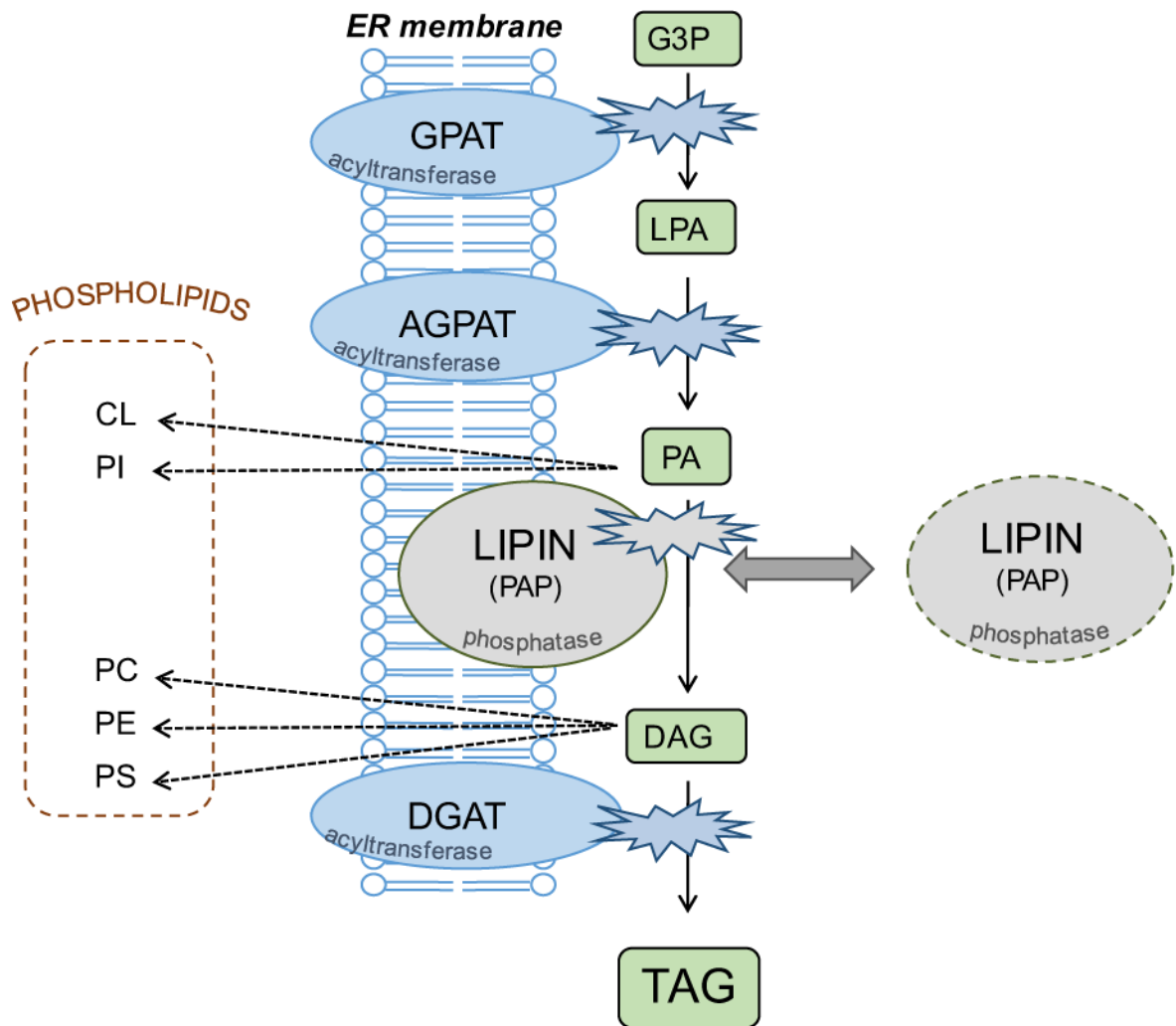
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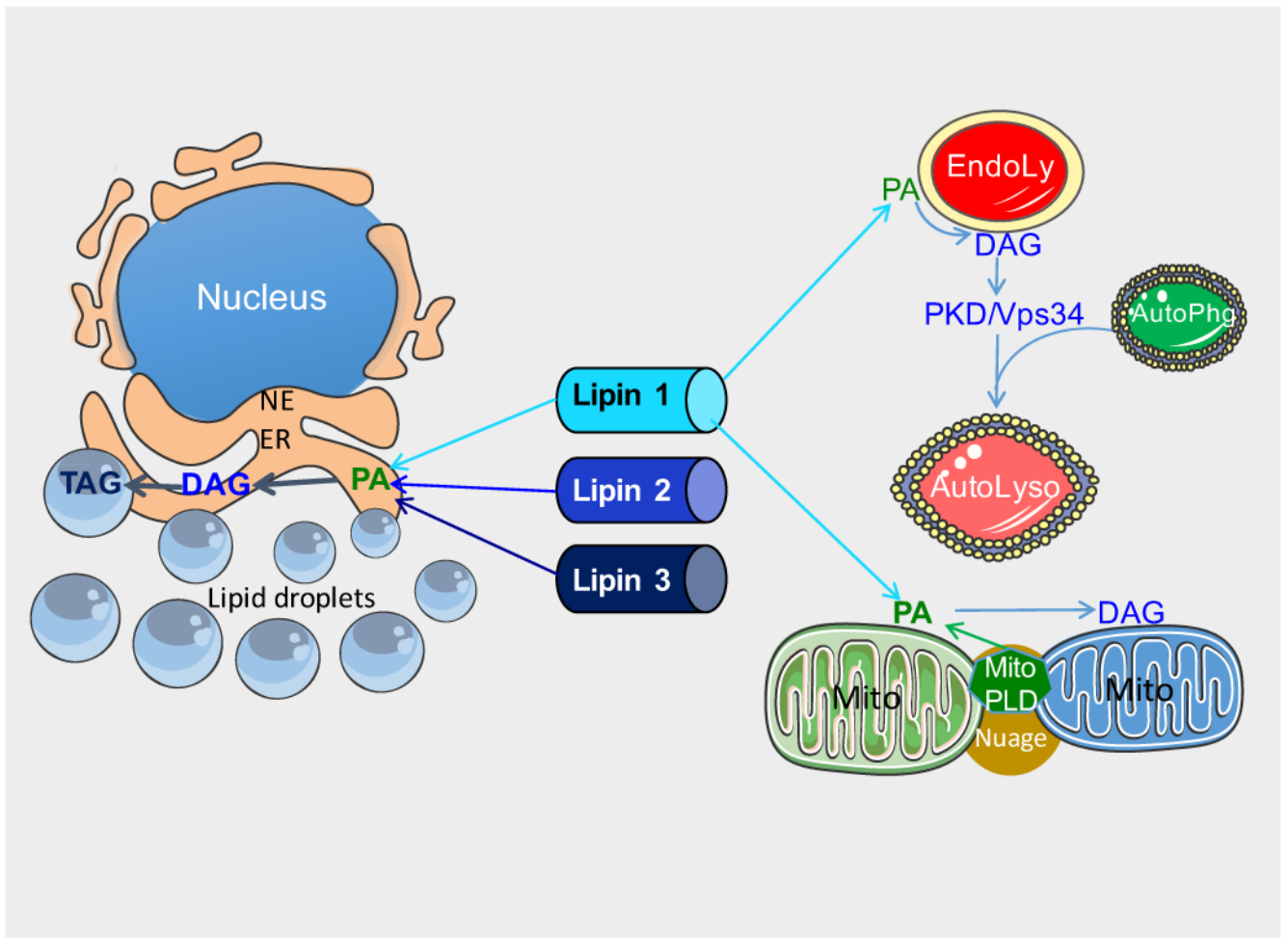
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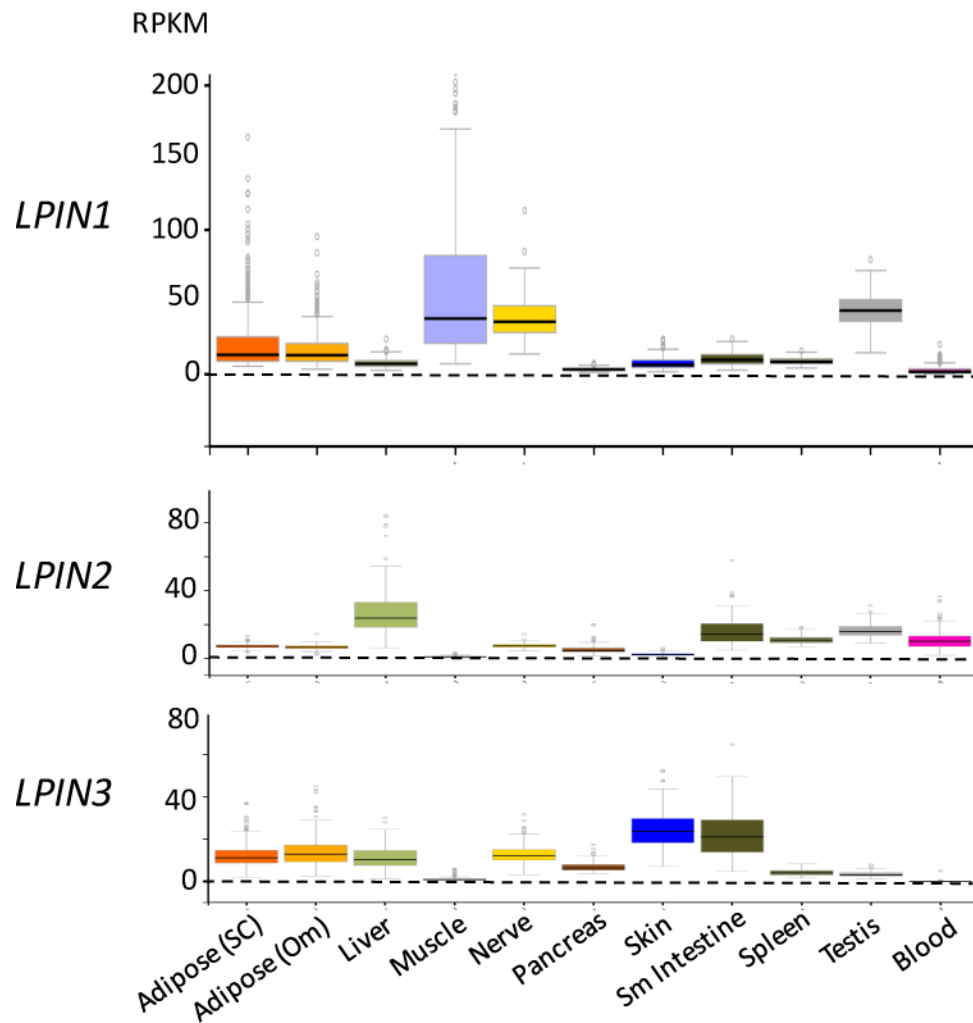


### Figure 1. Enzymes of the glycerol 3-phosphate pathway

The primary route for triacylglycerol synthesis in most mammalian tissues involves the sequential transfer of acyl CoAs to a glycerol 3-phosphate backbone by the action of acyltransferases (GPAT, AGPAT, DGAT), and removal of the phosphate group by the action of lipin phosphatidic acid phosphatases (PAP). This pathway also provides building blocks for zwitterionic and anionic phospholipids. The acyltransferases are membrane-resident proteins, whereas lipins may reside in the cytosol and transiently interact with membranes containing the lipid substrate, PA. GPAT, glycerol 3-phosphate acyltransferase; AGPAT, acylglycerol 3-phosphate acyltransferase; DGAT, acyl CoA:diacylglycerol acyltransferase; LPA, lysophosphatidic acid; PA, phosphatidic acid; DAG, diacylglycerol; TAG, triacylglycerol; CL, cardiolipin; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.



**Figure 2. Lipin localization to diverse organelles through association with membrane lipids**  
 Lipin proteins associate with membranes of several organelles, including the ER, endolysosomes, mitochondria, and lipid droplets. See text for description of mechanisms. EndoLy: late endosome or lysosome; AutoLyso: autolysosome; AutoPhg: autophagosome; ER: endoplasmic reticulum; Mito: mitochondria; NE: nuclear envelope; Nuage: Nuage pi-body; PA: phosphatidic acid; DAG: diacylglycerol; TAG: triacylglycerol; PDK/Vps34, protein kinase D/phosphatidylinositol-3 kinase; PLD, phospholipase D; MitoPLD, a phospholipase D family member found on mitochondria.



**Figure 3. Lipin mRNA expression levels in human tissues**

Data are from RNA-seq studies performed as part of the Genotype-Tissue expression Project [110]. RPKM, reads per kilobase of transcript per million mapped reads. Adipose (SC), subcutaneous adipose tissue; Adipose (om), omental adipose tissue; Sm intestine, small intestine; blood refers to white blood cells.