Minireview

# The lipin protein family: Dual roles in lipid biosynthesis and gene expression

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**Abstract** The prevalence of obesity in the western world has focused attention on factors that influence triglyceride biosynthesis, storage, and utilization. Members of the lipin protein family have a newly discovered enzymatic role in triglyceride and phospholipid biosynthesis as a phosphatidate phosphatase, and also act as an inducible transcriptional coactivator in conjunction with peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) coactivator-1$\alpha$ and PPAR$\alpha$. Through these activities, the founding member of the family, lipin-1, influences lipid metabolism and glucose homeostasis in diverse tissues including adipose tissue, skeletal muscle, and liver. The physiological roles of lipin-2 and lipin-3 are less well defined, but are likely to carry out similar functions in glycerolipid biosynthesis and gene expression in a distinct tissue distribution.

## 1. Introduction

It is now well established that adipose tissue plays a central role in metabolic homeostasis. Both excessive and inadequate adipose tissue mass are associated with conditions such as insulin resistance, diabetes, hyperlipidemia, and premature coronary artery disease [1–3]. The common thread in obesity and adipose-deficient conditions such as lipodystrophy is impaired adipocyte function. Adipocytes are the source of secreted factors such as leptin and adiponectin, which have important roles in modulating metabolism in the brain and peripheral tissues [4]. In particular, leptin acts in the hypothalamus to promote satiety, and in peripheral tissues to regulate energy expenditure and hepatic glucose production. Adiponectin increases glucose uptake in skeletal muscle, and also reduces hepatic glucose production and inflammation. In obesity and lipodystrophy, the levels and/or sensitivity to these and other adipokines may be reduced, contributing to insulin resistance. Lipodystrophy and obesity may also promote lipid accumulation in skeletal muscle, liver, and pancreatic beta cells, leading to impaired function of these tissues and impaired metabolic homeostasis [5].

The association of adipose tissue with many of the conditions underlying the metabolic syndrome has fueled the search for factors that influence adipocyte differentiation and lipid storage. Work performed in the last two decades has revealed a complex network of factors that regulate adipocyte differentiation through transcriptional and cell cycle control [6,7]. Likewise, biochemical and genetic studies have defined many of the key players in triacylglycerol (TAG) biosynthesis, storage, and lipolysis in adipose tissue [8]. In this review, we focus on the lipin family of proteins, which appear to have dual cellular roles, serving as an enzyme required for TAG and phospholipid biosynthesis, and as a transcriptional coactivator in the regulation of lipid metabolism genes. Through these activities, the lipin proteins, particularly lipin-1, appear to play important roles in metabolic homeostasis in adipose tissue as well as skeletal muscle, liver, and other cell types.

## 2. The lipin protein family

The founding member of the lipin protein family, lipin-1 was identified in 2001 by positional cloning of the mutant gene underlying lipodystrophy in the fatty liver dystrophy (*fld*) mouse [9]. The *fld* mouse is named for two key features of the mutant phenotype – the presence of a fatty liver for the first 2 weeks of the postnatal period, and the subsequent development of a peripheral neuropathy characterized by poorly compacted myelin sheaths, abnormal Schwann cells, and myelin breakdown [10,11]. The *fld* mouse also lacks normal adipose tissue depots throughout the body, including visceral and subcutaneous white adipose tissue, and interscapular brown adipose tissue [12]. This phenotype is reminiscent of some forms of generalized lipodystrophy in humans, but differs in two ways – fatty liver is typically chronic in human lipodystrophies, while

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it is transient in the *fld* mouse, and peripheral neuropathy occurs only in a subset of human patients [1].

The *fld* mouse is deficient for lipin-1 due to a null mutation in the *Lpin1* gene [9]. A second mutant allele carrying a missense point mutation arose independently in the *fld²ᴶ* mouse strain. This mutation converts an evolutionarily conserved glycine to arginine, and results in a similar phenotype to the null mutation [9]. Lipin-1 is expressed at highest levels in white and brown adipose tissue, skeletal muscle, and testis, and is also detectable in liver, peripheral nerve, brain, kidney, and pancreatic beta cells [9,13–15]. Expression of lipin-1 in peripheral nerve is apparent in both the endoneurium, in which Schwann cells are the predominant cell type, and in the peri/epineurium, which includes adipocytes [15]. These observations suggest that the neuropathy observed in *fld* mice is a result of local lipin-1 deficiency in the nerve.

In mammals, there are three members of the lipin gene family, which encode four lipin protein isoforms (Fig. 1A). Two lipin-1 protein isoforms are generated by alternative mRNA splicing of the *Lpin1* gene, giving rise to proteins with predicted sizes of approximately 98 and 102 kDa [13,16]. Lipin-2 and lipin-3 were identified through sequence similarity to lipin-1 (44–48% amino acid identity) [9]. The three mammalian lipin genes differ in their tissue expression patterns (Fig. 1A), suggesting that they have unique physiological roles. Thus, lipin-2 is most prominently expressed in liver and brain, whereas lipin-3 is present at low levels in several tissues, notably small intestine and liver [17]. At present, little is known about the physiological role of these two family members. However, the human lipin-2 gene, *LPIN2*, was recently identified as the mutant gene in Majeed syndrome, a disorder characterized by congenital dyserythropoietic anemia, recurrent fever, chronic recurrent ostoemyelitis, and cutaneous inflammation [18]. Family-specific *LPIN2* mutations were identified in two unrelated Jordanian families with Majeed syndrome, including an early termination mutation and a single amino acid substitution [19]. The normal physiological role of lipin-2 in tissues affected in Majeed syndrome is unclear, but the

phenotype of individuals harboring these mutations suggest a non-redundant role for lipin-2 function. Virtually nothing is currently known about the physiological function of lipin-3.

Lipin gene orthologs are also conserved across a broad range of non-mammalian species, suggesting a fundamental biological role [9]. Fish and plants appear to have two lipin-related genes; nematodes, fruit flies, plasmodium, and yeast each have one lipin gene ortholog. Lipin proteins in nearly all species possess nuclear localization signals and several putative serine and threonine phosphorylation sites. In addition, extended regions at the amino- and carboxy-terminal ends of the lipin proteins (the amino-terminal lipin (N-LIP) and carboxy-terminal lipin (C-LIP) domains) are highly conserved among all lipin proteins and all organisms, suggesting an important functional role for these domains (Fig. 1). Indeed, the C-LIP domain contains the active site motifs for the enzymatic and transcriptional coactivator functions of lipin-1 (Fig. 1B; described in a later section).

### 3. Lipin-1 is required for the development of mature adipocytes

Studies of lipin-1-deficient mice and cells have shown that lipin-1 is required for adipocyte differentiation. Differentiation occurs through an ordered cascade of gene-expression changes that convert preadipocytes to mature, lipid-loaded adipocytes [6,7]. Gene-expression analysis in lipin-1-deficient cells and adipose tissue from *fld* mice revealed a striking defect in the induction of the adipogenic transcription program. Lipin-1-deficient cells and tissues failed to induce expression of two key transcription factors, peroxisome proliferator-activated receptor γ (PPARγ) and CAAT enhancer binding protein-α (C/EBPα), and their downstream target genes [20]. Instead, they expressed high levels of preadipocyte factor-1, an inhibitor of adipogenesis. Complementation of lipin-1-deficient preadipocytes with a retroviral vector expressing PPARγ partially rescued adipocyte differentiation, suggesting that lipin-1 may be required upstream of PPARγ expression [20]. Consistent with this possibil-
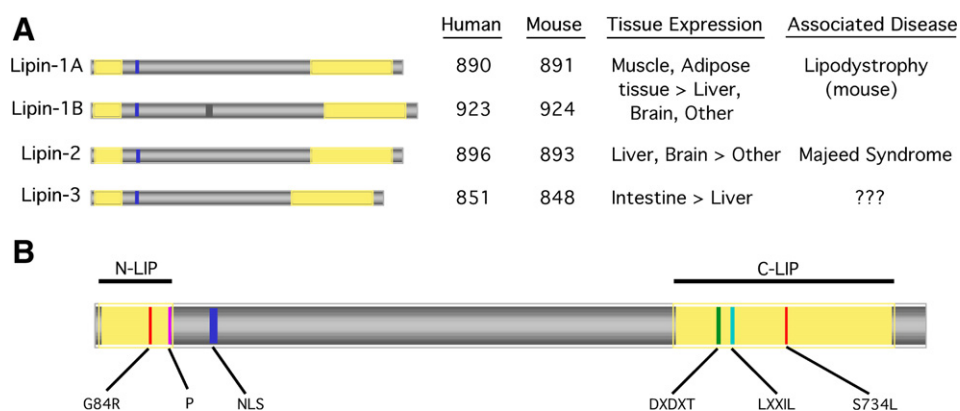


Fig. 1. The lipin protein family. (A) Four lipin protein isoforms are shown schematically, with regions of highest amino acid identity shown as yellow bars and nuclear localization signal as a blue line. Lipin-1A and -1B are alternative mRNA splice forms of the *Lpin1* gene, with an additional exon encoding 33 amino acids included in the lipin-1B protein (dark gray rectangle). The number of amino acid residues for human and mouse versions of each protein is shown. Tissues with prominent mRNA expression levels and known diseases resulting from mutation in the mouse *Lpin1* and human *LPIN2* genes are listed. (B) Known functional motifs and disease mutations in lipin proteins. N-LIP and C-LIP domains exhibit conservation between family members and in lipin orthologs from all species. NLS, nuclear localization signal; G84R, mutation in *Lpin1* that causes lipodystrophy; P, serine residue 106, which is known to be phosphorylated in response to insulin; DXDXT, PAP1 enzyme active site; LXXIL, transcription factor interaction domain; S734L, mutation in *LPIN2* that causes Majeed syndrome.

ity, during differentiation of the 3T3-L1 preadipocyte cell line, lipin-1 expression was induced at two time points. Lipin-1 was first expressed transiently between 10 and 20 h after induction of differentiation – before PPARγ is expressed. After returning to baseline levels, lipin-1 expression was induced again at 2 days after induction, and reached peak levels in mature, lipid-loaded adipocytes. This biphasic pattern suggests that lipin-1 has a role in establishing the adipogenic gene-expression program early during differentiation, and a later role in bringing about the mature adipocyte phenotype. These two roles may be related to the dual molecular functions of lipin, described in a later section.

In addition to the biphasic expression of lipin-1 in differentiating adipocytes, further complexity is conferred by the expression of the lipin-1A and lipin-1B protein isoforms from *Lpin1*. The isoforms appear to serve distinct functions in adipocytes, with unique expression dynamics, subcellular localization, and effects on gene expression [16]. As adipocyte differentiation progresses, lipin-1A levels diminish and lipin-1B levels increase. As a result, lipin-1B is the predominant form in mature adipocytes. Both isoforms can localize to the cytoplasm or to the nucleus; however, in mature adipocytes, lipin-1A is found predominantly in the nucleus, whereas lipin-1B tends to be cytoplasmic.

Lipin-1A and lipin-1B also have distinct effects on adipocyte gene expression. When retroviral expression vectors were used to complement lipin-1-deficient mouse embryonic fibroblasts, expression of lipin-1A led to the induction of adipogenic genes, such as PPARγ and C/EBPα [16]. In contrast, lipin-1B induced lipogenic genes, such as fatty acid synthase, stearoyl CoA desaturase-1, and diacylglycerol acyltransferase (DGAT). Lipin-1A also induced lipogenic genes, but to a lesser degree. Thus, the two isoforms appear to play complementary roles during the conversion of preadipocytes to mature, lipid-filled adipocytes.

## 4. Enhanced lipin-1 expression in transgenic mice causes obesity

While lipin-1 deficiency produces lipodystrophy, enhanced lipin-1 expression in transgenic mice promotes obesity. Lipin-1 constructs driven by adipocyte fatty acid binding protein (aP2) regulatory elements or muscle creatine kinase regulatory elements were used to produce transgenic mice with elevated lipin-1 expression in adipose tissue or skeletal muscle, respectively [21]. On a high-fat diet, both types of transgenic mice gained weight much faster than non-transgenic mice, despite equivalent food intake. However, the mechanism for the obesity and the effects on glucose homeostasis differed depending on whether expression was enhanced in adipose tissue or muscle.

The adipose-specific lipin-1 transgenic mice had a normal number of adipocytes, but increased fat cell triglyceride content and increased expression of lipogenic genes, including fatty acid synthase, acetyl-CoA carboxylase, and DGAT [16,21]. Unexpectedly, despite their higher body weight and fat content, these mice had lower glucose and insulin levels than non-transgenic mice, on both chow and high-fat diets. Although the mechanism is not known, one possibility is that more efficient fatty acid trapping in adipose tissue may reduce lipid deposition and compromised insulin action in muscle, liver, and other tissues.

The muscle-specific lipin-1 transgenic mice gained even more weight than adipose-specific transgenic mice. These mice became obese on a chow diet, and weight gain was accelerated further on a high-fat diet. After only 6 weeks on the diet, the muscle-specific transgenic mice had a 70% increase in body weight; the increase in non-transgenic mice was only 20% [21]. These transgenic mice had normal food intake, but a 15% reduction in energy expenditure and reduced fatty acid oxidation in muscle. Moreover, unlike adipose-specific lipin-1 transgenic mice, they developed insulin resistance, presumably due, in part, to increased triglyceride accumulation and altered metabolism in muscle. Other studies have also implicated lipin-1 in muscle metabolism. For example, lipin-1 expression in muscle is increased in conditions that cause muscle atrophy (e.g., diabetes, cachexia, uremia, and prolonged fasting) [22].

## 5. Lipin-1 levels in adipose tissue correlate with insulin sensitivity and reduced inflammatory cytokine expression in humans

The positive relationship between adipose tissue lipin-1 levels and insulin sensitivity that was originally observed in the aP2 transgenic mouse has been confirmed in two studies in humans. In a group of Finnish subjects, lipin-1 mRNA levels in adipose tissue were inversely correlated with glucose, insulin, and insulin resistance as measured by the homeostatic model assessment of insulin resistance index [23]. Furthermore, these studies demonstrated that specific polymorphisms in the *LPIN1* gene are associated with insulin levels and body mass index in dyslipidemic Finnish families and in a case-control sample of lean and obese individuals [23]. In a study of American subjects, insulin sensitivity measured with a frequently sampled glucose tolerance test confirmed that lipin-1 expression levels in adipose tissue are inversely correlated with insulin resistance [24]. Thus, high adipose tissue lipin-1 mRNA expression levels are associated with improved insulin sensitivity across species. In addition, allelic variants of *Lpin1* influence insulin levels and body mass index. However, unlike the studies in transgenic mice, studies in humans have not revealed a positive correlation between lipin-1 mRNA levels and body mass, perhaps because the effect is masked by other genetic or environmental factors, or the endogenous gene is subject to regulation that does not occur with the transgene.

Although the mechanism by which increased lipin-1 expression in adipose tissue leads to enhanced insulin sensitivity is not well understood, lipin-1 expression levels in cultured human adipocytes leads to enhanced expression of glucose transporter 4 (GLUT4) and increased adipocyte glucose uptake [25]. Interestingly, lipin-1 expression levels in human adipose tissue and in cultured adipocytes increase in response to treatment with thiazolidinedione drugs [24]. These results raise the possibility that enhanced lipin-1 levels are one effector of the insulin-sensitizing effects and/or increased adiposity associated with TZD treatment. Lipin-1 expression in adipose tissue is also enhanced by treatment with harmine, newly discovered to increase insulin sensitivity in vitro and in vivo [26].

Although no *LPIN1* mutations have thus far been identified in congenital human lipodystrophy, lipin-1 expression levels have been assessed in adipose tissue from patients with HIV-associated lipodystrophy [27]. In HIV patients, but not control subjects, lipin-1 mRNA levels in adipose tissue were positively correlated with limb fat mass, and lipin-1 expression levels

were lower in patients that had developed lipodystrophy. Lipin-1 mRNA levels were also inversely correlated with adipose tissue expression of inflammatory cytokines that increase in HIV-lipodystrophy, such as interleukin (IL)-6, IL-8, and IL-18. Thus, maintenance of higher lipin-1 levels in adipose tissue is associated with increased fat mass and reduced cytokine expression in HIV-associated lipodystrophy, and may therefore influence the overall metabolic status.

## 6. Lipin-1 is required for normal metabolic flux between adipose tissue and liver

Many conditions with compromised adipose tissue function, including lipodystrophy and obesity, are characterized by increased hepatic fat storage leading to chronic fatty liver. The lipin-1-deficient *fld* mouse is an exception in this regard, as the adult mice have lipodystrophy without fatty liver. The characterization of metabolism in the adipose tissue, liver and skeletal muscle of *fld* mice has shed some light on the possible mechanism, and revealed a role for lipin-1 in the coordination of peripheral glucose and fatty acid storage and utilization throughout the diurnal cycle [28]. As determined by indirect calorimetry and studies with stable-isotope-labeled substrates, the *fld* mouse was found to have an unusual adaptation to the lack of adipose tissue stores. During the fed state, *fld* mice store twofold greater amounts of glycogen in liver and muscle, and utilize these reserves in place of fat during the fasted state. During the feeding period, fatty acid biosynthesis is enhanced in the *fld* liver to provide energy substrates to peripheral tissues, thus sparing glucose for storage. These findings explain how *fld* mice survive fasting without triglyceride storage in their adipose tissue and why triglycerides do not accumulate in the liver. They also suggest that lipin-1 may normally play a role in directing nutrients toward energy storage or utilization. This is consistent with the observation

that lipin-1 is a downstream target of the mammalian target of rapamycin (mTOR), which functions at the interface between nutrient sensing and regulation of metabolic responses [13].

## 7. Lipins function as phosphatidate phosphatase enzymes in TAG and phospholipid biosynthesis

Studies described above have revealed important physiological roles for lipin-1 in metabolic homeostasis, but the cellular function of lipin proteins had remained mysterious until recently, when two distinct functions have been identified. The first is serving as an enzyme in TAG and phospholipid biosynthesis; the second is acting as a transcriptional coactivator that regulates expression of fatty acid utilization and lipid synthetic genes (Fig. 2). These two functions are described in the following two sections.

In most mammalian cell types, including adipocytes, TAG is synthesized via the glycerol phosphate pathway. Acylation of glycerol phosphate occurs through a step-wise addition of acyl groups, each addition catalyzed by a distinct enzyme (Fig. 2) [8]. The enzymes catalyzing most steps in TAG biosynthesis had been identified, but the molecular identity of phosphatidate phosphatase-1 (PAP1), which catalyzes the conversion of phosphatidate to diacylglycerol (DAG), was unknown until recently. DAG is the precursor for both TAG, through the action of DGAT enzymes, and for neutral phospholipids, phosphatidylcholine and phosphatidylethanolamine [29].

Carman's group purified PAP1 activity from *Saccharomyces cerevisiae* and through amino acid sequencing, identified it as Smp2p, the yeast ortholog of lipin [30]. The 3 mammalian lipin proteins were subsequently demonstrated to act as $Mg^{2+}$-dependent PAP1 enzymes, and to contain the DxDxT catalytic motif in the C-LIP domain (Fig. 1B), which is conserved from mammals to yeast [17,31,32]. The presence of the DxDxT mo-
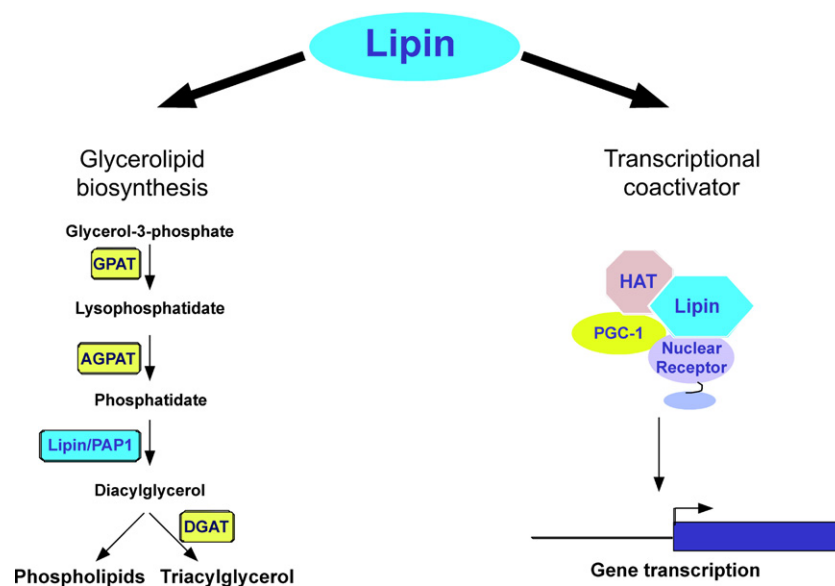


Fig. 2. Dual cellular functions of lipin proteins. Lipin-1, -2, and -3 all exhibit phosphatidate phosphatase activity, which plays a role in triglyceride and phospholipid biosynthesis. Lipin-1 has also been shown to act as a transcriptional coactivator in liver, directly interacting with nuclear receptors such as PPARα and the coactivator PGC-1α.

tif identifies the lipins as members of the haloacid dehalogenase (HAD)-like superfamily [33,34]. In the DxDxT catalytic motif, the first aspartate residue acts as a nucleophile that forms an aspartyl-intermediate during catalysis. The second acidic residue binds and protonates the substrate leaving group in the first step, and deptotonates the nucleophile of the second step. The $Mg^{2+}$ cofactor is coordinated by the carboxylate group of the first aspartate and the backbone of the second aspartate. Consistent with the requirement of the aspartate residues for catalytic activity, mutation of the first aspartate within the DxDxT motif (D712E) abolishes PAP1 activity of lipin-1 [31].

Using tissues from *fld* mice, it was determined that lipin-1 accounts for all PAP1 activity in white and brown adipose tissue, and skeletal muscle, the metabolic tissues with the highest levels of lipin-1 expression [17]. This is consistent with the impaired TAG accumulation in adipose tissue of *fld* mice, as well as the increased TAG accumulation in adipocytes of lipin-1 adipose-specific transgenic mice [12,21]. PAP1 activity was also absent in heart, lung, and kidney of *fld* mice [32]. Activity in liver of *fld* mice, however, was normal [17] or reduced by 50% [32], suggesting that perhaps another member(s) of the lipin protein family compensate. Consistent with these possibilities, lipin-2 is normally expressed at substantial levels in liver of wild-type and *fld* mice, and lipin-3 is upregulated in the liver of *fld* mice [17].

Expression of each of the lipin protein family members (lipin-1A, lipin-1B, lipin-2, and lipin-3) in cultured cells revealed that every member has PAP1 activity, and that activity is specific for phosphatidate, having no activity against other lipid phosphates including lysophosphatidic acid, ceramide-1-phosphate, or sphingosine-1-phosphate [17]. A comparison of lipin-1A and lipin-1B revealed approximately 70% higher PAP1 specific activity and higher $V_{max}$ for the longer isoform, lipin-1B [17]. Since the 33 amino acids that are unique to lipin-1B are spatially distant from the DxDxT catalytic motif, the activity difference is apparently related to other structural differences between the two isoforms. The $V_{max}$ for lipin-2 was similar to that of lipin-1A, while lipin-3 was somewhat lower. All three family members exhibited strong positive cooperativity for phosphatidate, suggesting that the lipins function as dimers or higher-order oligomers [17].

As described above, the DAG that results from PAP1 action is a substrate for phospholipid as well as TAG biosynthesis. To date, little work has directly assessed the physiological role of mammalian lipin proteins in phospholipid biosynthesis. However, elegant studies in yeast have shown that Smp2 regulates nuclear membrane growth during the cell cycle by controlling phospholipid biosynthesis [35]. Smp2-deficient cells undergo a massive expansion of the nuclear envelope, due to transcriptional upregulation of phospholipid biosynthetic gene expression. The resulting nuclear membrane extensions observed in Smp2-deficient yeast are reminiscent of membrane whorls observed in brown adipose tissue and liver of lipin-1-deficient *fld* mice [12]. Interestingly, recent studies have implicated lipin-1 in the increased phospholipid synthesis for endoplasmic reticulum membrane expansion that occurs as B lymphocytes differentiate into antibody-secreting plasma cells [36]. These studies raise the possibility that lipin-1, and potentially other lipin protein family members, have roles in additional conditions that involve membrane expansion.

## 8. Lipin-1 is an inducible transcriptional coactivator

As described above, lipin-1 is present both in the cytoplasm and in the nucleus of mammalian cells. Studies in both budding and fission yeast also suggest that lipin-1 has a specific function in the nucleus. Thus, mutation of *Ned1*, the lipin ortholog in fission yeast, causes aberrant nuclear shape and chromosome missegregation [37]. Ned1p also interacts with three nuclear proteins involved in the Ran GTPase cycle, which regulates nuclear envelope formation and directional nuclear transport. These interactions occur through the evolutionarily conserved N-LIP domain of Ned1p, suggesting that similar interactions might occur in mammalian lipin proteins. Furthermore, the regulation of phospholipid biosynthesis by Smp2 described above occurs through Smp2 interactions with promoters of phospholipid biosynthetic genes, suggesting a direct role in regulation of gene transcription [35].

Mammalian lipins may also have a role in regulation of gene expression. Recently, Finck et al. [31] showed that mammalian lipin-1 has transcriptional coactivator activity. In studies of PPARγ coactivator-1α (PGC-1α) knockout mice, these investigators uncovered a role for lipin-1 in activation of fatty acid oxidation genes during fasting. This occurs through direct interaction of lipin-1 with PGC-1α and PPARα, leading to the formation of a complex that modulates gene transcription. This interaction occurs through an α-helical leucine-rich motif (LxxIL) in the C-LIP domain of lipin-1 (Fig. 1B). Enhanced lipin-1 expression in hepatocytes amplified the expression of PGC-1α/PPARα target genes involved in fatty acid oxidation (e.g., acyl CoA oxidase, PPARα, carnitine palmitoyl transferase-1, medium and very long chain acyl CoA dehydrogenases), and suppressed expression of genes involved in de novo lipogenesis (e.g., sterol response element binding protein-1, fatty acid synthase, and stearoyl CoA desaturase) [31]. In addition to PPARα, lipin-1 was shown to interact with HNF4α, PPARβ, PPARγ, and the glucocorticoid receptor [31]. And although lipin-1 lacks a DNA binding domain, chromatin immunoprecipitation assays revealed interaction of lipin-1 containing complexes with the PPARα gene promoter, analogous to the interaction of Smp2 with phospholipid synthesis gene promoters in yeast [31,35]. Both lipin-2 and lipin-3 possess the LxxIL motif that mediates interaction with nuclear receptors, and initial studies suggest that lipin-3 can physically interact with PPARα [31]. The physiological role of the transcriptional coactivator function of lipin family members in tissues other than liver remains to be determined.

## 9. Regulation of lipin-1 transcription, phosphorylation, and activity

It appears that regulation of lipin-1 activity is complex, occurring at the levels of mRNA transcription, mRNA splicing, protein phosphorylation, and subcellular localization. The regulation of PAP1 activity has been studied for more than 25 years, prior to the identification of lipin proteins. Unlike most TAG biosynthetic enzymes, which reside in the ER or mitochondria, PAP1 is primarily cytosolic and translocates to the ER membrane in response to fatty acids or acyl-CoAs [38]. In liver, PAP1 activity is increased in diabetes, hypoxia, and toxic fatty liver and in response to elevated glucocorticoids

[39]. In adipose tissue, however, PAP1 activity is decreased in starvation and diabetes, reflecting decreased TAG synthesis and increased lipolysis [40]. Thus, there appear to be important differences in the regulation of PAP1 activity in specific tissues.

The identification of lipin-1 as PAP1 has allowed initial studies of its regulation at the transcriptional and post-transcriptional levels. It has been known for several years that PAP1 is regulated by phosphorylation [38], and there have now been multiple sites identified on the lipin-1 protein that are phosphorylated in response to insulin [13,32]. Three of the phosphorylation sites identified in lipin-1 ($Ser^{106}$, $Ser^{634}$, and $Ser^{720}$) are conserved in all three lipin family members, and in the yeast lipin ortholog. $Ser^{106}$ appears to be a major site of insulin-stimulated phosphorylation, and this phosphorylation is dependent on the mTOR pathway, as demonstrated by rapamycin inhibition [13,32]. In contrast to insulin, epinephrine and oleic acid both lead to lipin-1 dephosphorylation [32]. Importantly, phosphorylation of lipin-1 does not appear to directly affect PAP1 activity, but rather to mediate the cytopasmic vs. microsomal localization, with the ratio of soluble to microsomal lipin-1 increased by phosphorylation. In the yeast, the enzymes responsible for Smp2 phosphorylation (Cdc28/Cdk1) and dephosphorylation (Nem1/Spo7) have been identified [35].

As described earlier, lipin-1 mRNA expression is regulated during adipocyte differentiation [16,20], and in liver during fasting and in type 1 and type 2 diabetes mellitus [31]. Hepatic lipin-1 is also regulated by dietary fatty acids, with a 400-fold increase in mRNA observed in response to a low fat diet containing saturated vs. polyunsaturated fats [41]. The regulation of lipin-1 by fatty acids appears to be mediated by PPARα, as it did not occur in PPARα deficient mice. In the mouse, lipin-1 gene expression is also under circadian regulation, with a striking peak in both liver and adipose tissue during the daytime [42], in agreement with the observed increase during fasting. The circadian regulation of lipin-1 in peripheral tissues may be under the control of endogenous glucocorticoids, as the circadian cycling of lipin-1 was abolished in adrenalectomized mice [43]. At present it is known that alternative splicing of lipin-1 mRNA is regulated by changes that occur during adipocyte differentiation [16], but the mechanism is not known.

## 10. Summary and future perspectives

Studies of the lipin protein family thus far have largely focused on the founding member of the family, lipin-1. Studies in mouse models and humans indicate an important physiological role for lipin-1 in tissues that function in lipid metabolism (adipose tissue, skeletal muscle, and liver) as well as other specialized cell types (Fig. 3). Lipin-1 levels in adipose tissue profoundly affect fat mass in mouse models and correlate positively with insulin sensitivity in mice and humans. A key advance has been the recent discovery of dual cellular functions of lipin-1 – PAP1 enzymatic activity and transcriptional coactivator activity. Although uncommon, this dual enzymatic-coactivator activity is not unique to lipin-1. Other metabolic enzymes with demonstrated transcriptional coactivator activities include glyceraldehyde-3-phosphate dehydrogenase [44] and *O*-GlcNac transferase [45]. Many exciting questions remain to be answered. How are the enzymatic and transcriptional activities of lipin-1 coordinated in the cell and in
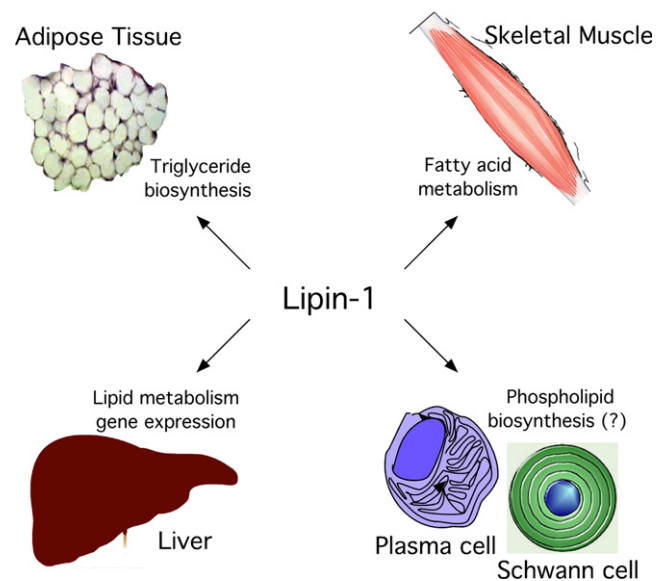


Fig. 3. Physiological roles of lipin-1. Lipin-1, and potentially other members of the lipin protein family, have important functions in lipid and energy homeostasis in adipose tissue, skeletal muscle, liver and other tissues.

different tissue types? What are the physiological roles of lipin-2 and lipin-3? Lipin-2 and lipin-3 possess PAP1 activity; do they also have second functions as transcriptional coactivators? An understanding of the function of lipin family members may lead to novel approaches for modulating important metabolic processes, such as triglyceride and phospholipid biosynthesis, and insulin sensitivity.

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