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Review

Lipin — The bridge between hepatic glycerolipid biosynthesis and lipoprotein metabolism

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ABSTRACT

Growing evidence links the three mammalian lipin proteins, *i.e.*, lipin-1, lipin-2 and lipin-3, to metabolic and cardiovascular diseases such as noninsulin-dependent diabetes mellitus and atherosclerosis. Lipin proteins play a dual function in lipid metabolism by acting as phosphatidate phosphatase (PAP) enzymes and as transcriptional regulators. Genetic variants within the human *LPIN1* and *LPIN2* genes are associated with metabolic syndromes. The fatty liver dystrophy (*fld*) mice carrying mutations within the *Lpin1* gene display life-long deficiency in adipogenesis, insulin resistance, neonatal hepatosteatosis and hypertriglyceridemia, as well as increased atherosclerosis susceptibility. Cell culture studies show that hepatic lipin-1 expression is selectively stimulated by glucocorticoids and repressed by insulin, and its subcellular localization governs the assembly and secretion of very low density lipoproteins (VLDL). In noninsulin-dependent diabetes, glucocorticoid signals lead to dyslipidemia characterized by overproduction of VLDL and atherogenic remnants. This puts lipin-1 as a key integrator of hormonal signals to the liver in diabetic dyslipidemia. This review summarizes the current understanding of the role that hepatic lipin-1 plays in the synthesis, storage and compartmentalization of glycerolipids, and highlights the lipid metabolic consequences associated with dysregulated lipin expression.

1. Introduction

The assembly and secretion of triacylglycerol (TAG)-rich VLDL represents a key component of hepatic TAG homeostasis and is tightly regulated by biosynthesis and availability of various lipid constituents including phospholipids and TAG [1,2]. The assembly of lipid constituents into VLDL particles requires the structural protein apolipoprotein (apo) B-100 [3], and this process is facilitated by the microsomal triglyceride transfer protein (MTP) located within the lumen of endoplasmic reticulum (ER) and Golgi apparatus [4]. Factors affecting VLDL assembly/secretion include lipid substrate availability (or lipid cargo) as well as the functionality and integrity of the ER/Golgi membranes (or lipid conveyor). Because of a dual function that lipin proteins play in both the biosynthesis of glycerolipids (by possessing PAP enzymatic activity) and in regulating the expression of genes involved in lipid metabolism (by acting as a transcription coactivator), and because of their localization at various intracellular compartments critical for membrane biogenesis, regulation of lipin expression represents a key component in the process of hepatic VLDL assembly and secretion. The three mammalian lipin family members, namely lipin-1, lipin-2 and lipin-3 are encoded by their respective genes. Mutations in the *Lpin1* gene in *fld* mice encumber lipin-1 function and cause a life-long deficiency in adipogenesis, neonatal hypertriglyceridemia and hepatosteatosis, as well as insulin resistance and increased susceptibility to atherosclerosis [5–8]. Intriguingly, lipin-1 deficiency in humans is not associated with lipodystrophy as mutations in the human *LPIN1* gene result in defective muscle energy metabolism, recurrent rhabdomyolysis and myoglobinuria in early childhood [9], which might be caused by a defect in fatty acid oxidation. However, genetic variants within human *LPIN1* [10–12] and *LPIN2* [13] genes are associated with phenotypes characteristic of metabolic syndromes. The current review highlights a function for lipin-1 as an integrator of hormonal signals to the liver, as well as a multifaceted bridge between hepatic glycerolipid biosynthesis and TAG-rich lipoprotein assembly/secretion under various metabolic conditions. Emphasis is centered on the expression, compartmentalization and post-translational modifications of lipin-1, as well as on the contribution of lipin-1 driven glycerolipid homeostasis to both the cargo and the conveyor aspects of TAG-rich lipoprotein assembly/secretion.

2. Dual function of lipin proteins in hepatic lipid homeostasis

The *Lpin1* gene was identified as the mutated gene underlying lipodystrophy in the two fatty liver dystrophy (*fld*) mouse strains BALB/cByl-*fld* (commonly known as *fld*) and C3H/HeJ-*fld*^{2J} [8]. The mouse *Lpin1* gene undergoes alternative mRNA splicing to generate two isoforms, namely lipin-1α and lipin-1β (Fig. 1) that are 891 and 924 amino acids in length, respectively [14]. The human *LPIN1* gene is also expressed in two isoforms (lipin-1α and lipin-1β) by alternative mRNA splicing [15], with a third splice variant (lipin-1γ) recently identified [16]. The human lipin-1γ specific sequence consists of 26 amino acids,

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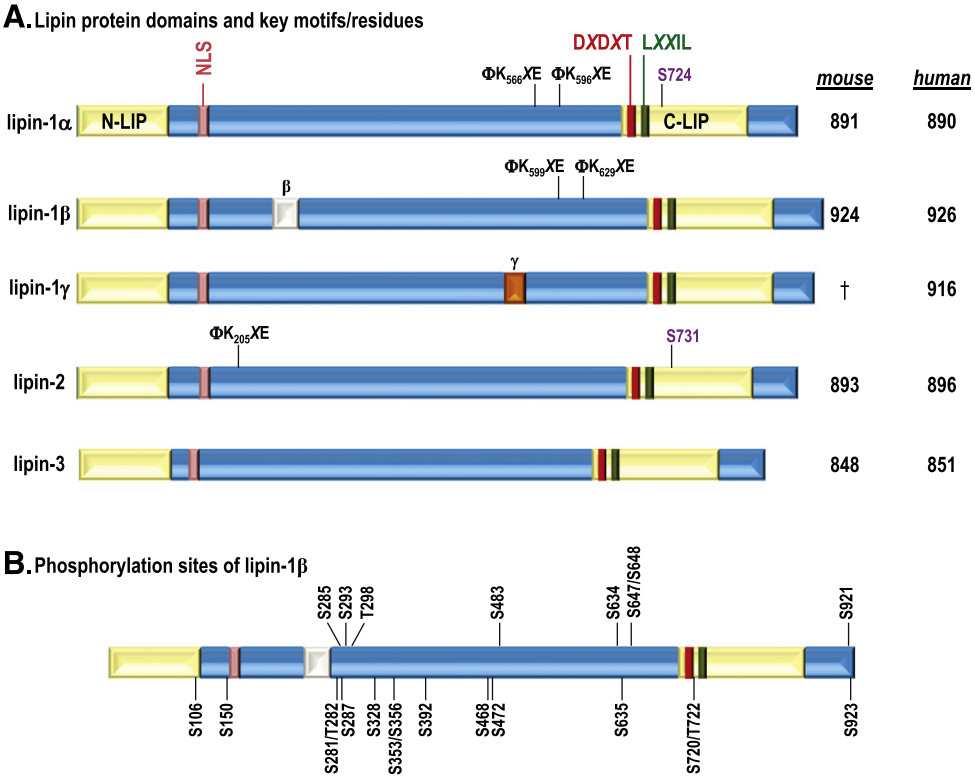

Fig. 1. Structural and functional motifs of lipin. A) Schematic diagram depicting mammalian lipin proteins (lipin-1α, lipin-1β, lipin-1γ, lipin-2 and lipin-3) with evolutionarily conserved N- terminal (N-LIP) and C-terminal (C-LIP) domains, a nuclear localization signal (NLS), phosphatidate phosphatase-1 (PAP) enzymatic motif (DXDXT), nuclear receptor interaction motif (LXXIL) and sumoylation (ΦKXE) consensus motifs (identified by Liu and Gerace [39]) indicated. *X* denotes any amino acid; Φ represents hydrophobic amino acids; β and γ denote the lipin-1β and lipin-1γ specific sequences, respectively. A conserved serine residue demonstrated to be required for PAP activity [30] in mouse lipin-1α (S724) and lipin-2 (S731) is also indicated. The number of amino acid residues for the mouse and human lipin-1α, lipin-1β and lipin-1γ isoforms is shown. † indicates the mouse counterpart of the human lipin-1γ specific sequence with 77% identity in deduced amino acids [16]. B) Phosphorylation sites of lipin-1β as identified by Harris et al. [22]. Residues S281/T282, S353/S356, S647/S648 and S720/T722 indicate pairs in which either residue may be phosphorylated [22].

and homology search against the mouse genome identified a counterpart of the human lipin-1γ specific exon with 77% identity in deduced amino acids [16]. Moreover, the human lipin-1β specific sequence comprises 36 amino acids, and sequence alignment shows 72% identity in deduced amino acids to the mouse counterpart [16]. Two other members of the lipin protein family, namely lipin-2 and lipin-3, are shown to share 44–48% amino acid sequence similarity to lipin-1 [8,17]. Mammalian lipin proteins gained biological appreciation with the discovery that they encode glycerolipid biosynthetic activity and transcriptional regulatory functions in lipid metabolism as outlined below.

2.1. Lipin encodes phosphatidate phosphatase activity

The *de novo* TAG synthesis pathway involves successive acylation of glycerol-3-phosphate to generate phosphatidate (PA) [18], which is then dephosphorylated by PAP to form diacylglycerol (DAG). Although it was known for a long time that the PAP-derived DAG is a key substrate for the biosynthesis of TAG, phosphatidylcholine (PC) and phosphatidylethanolamine [19–21], the molecular identity of PAP was unknown until it was unveiled in *Saccharomyces cerevisiae* in 2006 to be encoded by lipin (the yeast ortholog being Pah1p/Smp2p) [22]. It is now clear that all mammalian lipin proteins (*i.e.*, lipin-1α, lipin-1β, lipin-1γ, lipin-2 and lipin-3) possess PAP activity that is dependent upon Mg^{2+} or Mn^{2+} and on PA as a substrate [16,23–25]. Orthologs of lipin exist in plants, invertebrates, and single cell eukaryotes [8]. The PAP activity is conferred by the DXDXT catalytic motif (*X* can be any amino acid), which is commonly found in haloacid dehalogenase (HAD)-like superfamily of phosphatases [26–28]. The DXDXT motif is conserved from mammalian lipin-1 to yeast Pah1p/Smp2p [8], and it is present within the C-terminal end of the protein (termed C-LIP domain) (Fig. 1) in most lipin homologs. The first and second aspartate residues within the DXDXT motif are required for PAP activity in mouse lipin-1 [25] and yeast Pah1p/Smp2p [29]. Imbedded in the C-LIP domain also is an α-helical leucine rich motif (LXXIL) (Fig. 1) that mediates interaction with transcription factors [8,25], and site directed mutagenesis in this motif results in impaired PAP activity [25]. Furthermore, the conserved serine residue within the C-LIP domain, downstream of the PAP activity motif and the transcriptional coactivator motif (Fig. 1), has been shown to be required for PAP activity in lipin-1 and lipin-2 but not for the transcriptional coactivator functions [30].

The three lipin genes and their splice variants in mice and humans have distinct yet overlapping tissue specific expression patterns [24,30]. The highest level of lipin-1 expression occurs in white/brown adipose tissue, skeletal muscle, cardiac muscle and testis, with low expression in the liver, kidney, and brain. Lipin-1 is also expressed in Schwann cells of peripheral nerves, and its deficiency in these cells causes peripheral neuropathy in the *fld* mouse [31,32]. Lipin-2 is highly expressed in the liver [24,30], and hepatic lipin-2 mRNA levels are substantially higher than lipin-1 [30]. Lipin-2 is also expressed in the kidney, lungs, brain, gastrointestinal tract, salivary glands, circulating red blood cells and lymphoid tissues [30]. Lipin-3 is expressed at much lower levels with mRNA detectable in the liver and gut [24,30].

The contribution of each lipin isoform to PAP activity in liver cells is unclear, although it is known that lipin-1 accounts for all of the measured PAP activity in white/brown adipose tissues and skeletal muscle cells [24]. The lipin-1 deficient *fld* mice exhibit reductions in adipose tissue mass and cellular lipid content [7], which resemble the abnormalities associated with severe lipodystrophy in humans [33]. The *fld* mice also develop glucose intolerance, insulin resistance, and become

susceptible to atherosclerosis [7]. Lipin-1 overexpression increases TAG accumulation in adipocytes of adipose-specific lipin-1 transgenic mice [7,34]. However, lack of lipin-1 expression in the liver of *fld* mice translates into severe hypertriglyceridemia (as high as 1,000 mg/dl) and massive hepatosteatosis during the suckling period, with lipin-1 deficient hepatocytes exhibiting normal or half of the measured PAP activity [5–8,23,24,35]. Paradoxically, the hypertriglyceridemia and hepatosteatosis in *fld* mice resolve prior to weaning (between postnatal days 13–15), even though the mice maintain ~25% lower body weight [5,36]. The substantial hepatic PAP activity in *fld* mice could be attributable to compensatory upregulation of lipin-3 expression [24,30] and also due to substantial expression of hepatic lipin-2 [35]. Recent shRNA-mediated experiments in *fld* hepatocytes suggest that knocking down lipin-2 expression markedly diminishes hepatic PAP activity and suppresses TAG synthesis under high fatty acid supplementation conditions [35]. Thus, the expression of hepatic lipin-2 may account for significant PAP activity in the liver under lipin-1 deficiency (*fld*) conditions. However, in HeLa cells where lipin-1 expression is normal, knockdown of lipin-2 results in increased PAP activity, apparently as a result of compensatory upregulation of lipin-1 [37]. The relative contribution of different lipin proteins towards the PAP activity is, therefore, cell type specific.

2.2. Lipin acts as a transcriptional coactivator of lipid metabolism genes

Although lipin proteins do not contain DNA binding domains, a signature motif (LXXIL) (Fig. 1) located within the C-LIP domain [8] endows lipin proteins with the ability to interact with nuclear receptors and function as transcriptional regulators [17,38,39]. For instance, lipin-1 forms a complex with proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) and PPARα to enhance gene expression. Lipin-1 can also interact *in vitro* with nuclear receptors such as hepatocyte nuclear factor-4α (HNF-4α) and glucocorticoid receptor (GR) [25]. In addition, lipin-1 also induces the expression of key adipogenic transcription factors including PPARγ and C/EBPα [40]. Specifically, lipin-1α and lipin-1β appear to exert complementary roles in adipocyte differentiation. While lipin-1α induces the expression of adipogenic transcription factors, lipin-1β induces the expression of lipid synthesis genes encoding, for example, fatty acid synthase (FAS) and diacylglycerol acyltransferase (DGAT) [14]. In *fld* liver, lipin-1 deficiency results in the activation of the sterol regulatory element binding protein 1 (SREBP-1) and its target genes [36], as well as in very high expression levels of stearoyl-CoA desaturase-1 (*Scd1*) and apoA-IV (*Apoa4*) [41]. Additionally, acute lipin-1 deficiency in the mouse liver abolishes fasting-induced activation of *Ppara* and several PPARα/PGC-1α target genes, such as *Acadvl*, *Acadm* and *Fabp1*, that are involved in fatty acid β-oxidation [25]. The transcriptional coactivator activity of lipin-1α toward PGC-1α in neuronal cells is achieved through association and activation of myocyte enhancer factor 2 (MEF2), a transcriptional factor required for neuronal cells survival and differentiation [42]. However, the mechanisms by which lipin-1α activates MEF2 and its downstream target genes remain elusive. PGC-1α stimulates its own promoter by coactivating MEF2 [43], as well as lipin-1 expression [25]. It is suggested that lipin-1α employs sumoylation as a molecular switch to amplify the PGC-1α-MEF2 loop in neuronal cells [42].

Performing bioinformatic analysis of published gene expression profiling data [25] reveals a set of transcription factors that, we postulate, may be involved in modulating hepatic gene expression programs in response to lipin function (Table 1). Specifically, DNA binding sites preferred by these transcription factors are profoundly over-represented within the regulatory regions of genes that showed upregulation upon lipin-1 overexpression. In addition to PPARα that may interact with lipin-1 either directly or indirectly through PGC-1α, we found that lipin-1 may also exert some of its effects through HNF4α and SREBP. All of these transcriptional regulators are known to play a role in biological processes related to lipid homeostasis (Table 1). Noteworthy, lipin-1 is an inducible amplifier of the PGC-1α/PPARα signaling pathway, and PGC-1α is a coamplifier of lipin-1 in the liver. The glucocorticoid dexamethasone and cAMP increase mRNA levels of lipin-1 and PGC-1α in the liver [44], and lipin-1 interacts *in vitro* with HNF4α [25]. Partnering between PGC-1α and HNF4α is shown to be crucial for hepatic lipoprotein metabolism. Forced expression of PGC-1α in mouse and human hepatoma cells increases the mRNA of *Apoa4*, *Apoc2* and *Apoc3*, which have been implicated in VLDL and TAG metabolism. Moreover, adenoviral-mediated expression of PGC-1α into live mice stimulates the expression of these apolipoproteins and increases serum and VLDL TAG levels, a phenotype that is antagonized by PGC-1α knockdown [45]. While some of these transcription factors may function in a PGC1-dependent manner, others are thought to function independently of PGC-1 (Fig. 2). The LXXIL motif also occurs within lipin-2 and lipin-3. Transcriptional coactivator activity in concert with PGC-1α/PPARγ is shown with lipin-2 [30], and a physical interaction is reported between lipin-3 and PPARα [25]. Whether or not any of the lipin isoforms can act in PGC1-independent manner remains to gain experimental validation.

A function for lipin-1 in phospholipid biosynthesis and nuclear membrane biogenesis is also endorsed experimentally at the transcriptional coactivation level. In yeast, transcriptional regulation of phospholipid synthesis genes such as INO1 (which encodes inositol-3-phosphate synthase, the rate limiting enzyme for phosphatidylinositol synthesis) and OPI3 (which encodes the enzyme catalyzing the final steps in PC biosynthesis [46]) is controlled by the interaction of the Opi1p repressor with the Ino2p activator, which forms a complex with Ino4p that binds the yeast inositol-sensitive upstream activating sequence found in promoters of phospholipid synthesis genes. The function of Opi1p is governed by its interaction with PA (the PAP reaction substrate) at the nuclear/ER membrane. When PA levels are high (due to loss of PAP activity), Opi1p binds to PA at the ER membrane and transcription of phospholipid synthesis genes is derepressed. When PA levels are reduced (due to PAP activation), Opi1p translocates into the nucleus where it binds to Ino2p to repress transcription of phospholipid biosynthetic genes. A nuclear/ER membrane-localized phosphatase complex consisting of Nem1p–Spo7p recruits phosphorylated Pah1p/Smp2p onto the membrane. At the membrane, the phosphatase complex dephosphorylates Pah1p/Smp2p and allows the enzyme to interact with the membrane and bind to PA to catalyze the PAP reaction. Loss of PAP activity and inhibition of Smp2p/Pah1p dephosphorylation result in derepression of phospholipid biosynthetic genes and nuclear membrane expansion [29,82]. The significance between lipid biosynthetic activity and transcriptional regulation in hepatic lipid metabolism and homeostasis will be dissected in subsequent sections.

3. Regulation of lipin expression and compartmentalization

3.1. Correlation between hepatic VLDL secretion and lipin expression

Existing experimental evidence suggests that hepatic VLDL synthesis and secretion is highly influenced by the expression of lipin-1. Expression of lipin-1 is regulated by various physiologic and pathophysiologic stimuli that are known to exert an effect on lipoprotein metabolism in the liver. The level of hepatic lipin-1 expression is low in adult mice in the fed state [8,24], and lipin-1 contributes relatively little to total hepatic PAP activity [23,24]. However, the expression of lipin-1 is markedly upregulated under stress conditions. It has been known for a long time that glucocorticoids induce hepatic PAP activity and TAG synthesis, an effect that is synergized by glucagon and antagonized by insulin [48,49]. This glucocorticoid effect is consistent with the observed increase in hepatic PAP activity under stress conditions such as partial hepatectomy [50], starvation [51] and diabetes [52], as well as in response to

Table 1
Partial list of transcription factors whose preferred DNA binding sequences are especially abundant among lipin-1 modulated genes*.

Family name	Representative transfac PWMs	Possible binding factors	Connection to hepatic lipogenesis and VLDL assembly/secretion
PGC-1α or PGC-1β dependent			
Peroxisome proliferator-activated receptor (PPAR), hepatocyte nuclear factor 4 (HNF4)	M00134-V$HNF4_01-HNF-4alpha1	HNF4α, PPARα, PPARγ	HNF4α regulates transcriptionally MTP [129,130] and VLDL secretion [131,132]. PPARα induces MTP expression [133,134] TAG mass in hepatocytes [135]. Foxa2/PGC-1β complex induces MTP expression and VLDL secretion [136,137]. PPARγ ligand agonists suppress the human MTP promoter activity [130].
Nuclear receptor	M00192-V$GR_Q6-GR	GR, Estrogen Receptor, AR, PXR, ERR	Regulation of the *Lpin1* gene expression by glucocorticoids [58], and the GR indirectly stimulates VLDL receptor gene transcription in 3T3-L1 cells [138]. Estrogens modulates apoB100-containing lipoprotein metabolism [139,140]. Activation of PXR induces hepatosteatosis [141] and VLDL/LDL production [142]. ERRα with PGC-1α activate the apoA-IV via interaction with the apoC-III enhancer, as well as intestinal lipid transport [143].
PGC-1 independent			
Sterol response element binding protein	M00220-V$SREBP1_01-SREBP-1	SREBP1	SREBP1 regulates the *LPIN1* gene expression in human hepatoblastoma cells [68]. SREBP1 stimulates hepatic lipogenesis and VLDL secretion [144]. LXR-SREBP1c axis controls VLDL particle size [145] and production [146].
cAMP responsive element binding protein (CREB)	M00916-V$CREB_Q2_01-CREB	CREB1, XBP-1, ATF1, ATF2	Regulation of hepatic lipogenesis by the transcription factor XBP-1 [147]. Deletion of XBP1 in adult liver results in hypotriglyceridemia [148]. ATF-2 Ttansactivates the apoC-III promoter in hepatic cells [149], and apoC-III enhances hepatic VLDL assembly/secretion under lipid-rich conditions [108,150].
Aryl hydrocarbon receptor (AhR), hypoxia inducible factor (HIF)	M00976-V$AHRHIF_Q6-AhR	AhR, Arnt, HIF1α	Ahr affects serum TAG levels [151].
Signal transducer and activator of transcription (STAT)	M00497-V$STAT3_02-STAT3	STAT-1, STAT-3, STAT-5	STAT5b enhances the transcriptional activity of HNF4α towards apoC-III [152]. Jak/STAT dependent pathway stimulates SREBP-1c maturation [153]. Hepatic Stat-3 inactivation results in hypertryglyceridemia and hepatosteatosis [154].
Upstream stimulatory factor (USF)	M00726-V$USF2_Q6-USF-1-USF-2	USF1 and USF2	USF1 and USF2 stimulate hepatic lipase expression in HepG2 liver cells [155]. USF1 is linked to hyperlipidaemia and the metabolic syndrome [156].

*The dataset of Finck et al. [25] was downloaded from GEO (accession GSE5538) and normalized gene expression values were calculated. Genes whose expression is induced more than 2-fold (265 genes in total) were further analyzed. The sequence of their promoter region, from 1500 base pairs upstream of their transcription start site to 500 base pairs downstream of the start site (2 kb total), was subjected to position weight matrix (PWM) scans. The Cisgenome program was used to scan these 265 promoters for all vertebrate PWMs from the Transfac database. As background set, a group with 50 times as many promoters of the same length (13,250 promoters of 2 kb in total) was randomly selected, and was also subjected to PWM scan. PWM enrichment values among the test set of genes were calculated using hypergeometric distribution test: for a given PWM, the number of hits among the lipin-1 modulated promoters and among the control set of promoters, as well the size in base pairs of each promoter set were used as parameters. A cut-off of $p<0.005$ for the value of (1-cumulative probability) was used to identify significant PWMs. Abbreviations: AR, androgen receptor; ERR, estrogen-related receptor; GR, glucocorticoids receptor; PGC-1, proliferator-activated receptor-γ (PPARγ) coactivator-1; PXR, pregnane × receptor.

dietary fat/carbohydrate modifications or ethanol consumption [53,54]. In particular, the glucocorticoid dexamethasone stimulates TAG synthesis and secretion as VLDL particles [55–57]. This stimulatory effect of dexamethasone is associated with a specific induction of lipin-1 expression since lipin-2 or lipin-3 mRNA concentrations and protein levels are unchanged in mouse or rat primary hepatocytes under the same treatment [44,58]. The *cis* DNA sequence element responsible for the binding of the glucocorticoid receptor has been identified upstream of the promoter region of the *Lpin1* gene [58]. Our unpublished differential display PCR data reveals that in primary rat hepatocytes treated with dexamethasone, lipin-1 is one of two lipid synthesis genes (the other being HMG-CoA reductase) whose mRNA concentrations are drastically increased. The dexamethasone-induced effect on lipin-1 expression is synergized by cAMP and antagonized by insulin. Glucagon, through cAMP, increases the half-life of the dexamethasone-induced effect on hepatic lipin-1 expression and its encoded PAP activity [44], with cAMP modulating PAP activity through phosphorylation [48,59]. Insulin modulates phosphorylation of lipin-1 [23,60,61], its subcellular compartmentalization [23,61] and its expression [44,62]. Upregulation of lipin-1 expression under stress conditions (*e.g.*, glucocorticoid treatment or diabetes) may protect the liver from fatty acid overload and prevent lipotoxicity by converting fatty acids into TAG for secretion as VLDL particles or for storage in cytoplasm [48,62].

Work with hepatocytes isolated from fasting rats has shown that secretion of apoB and TAG-rich VLDL are influenced profoundly by

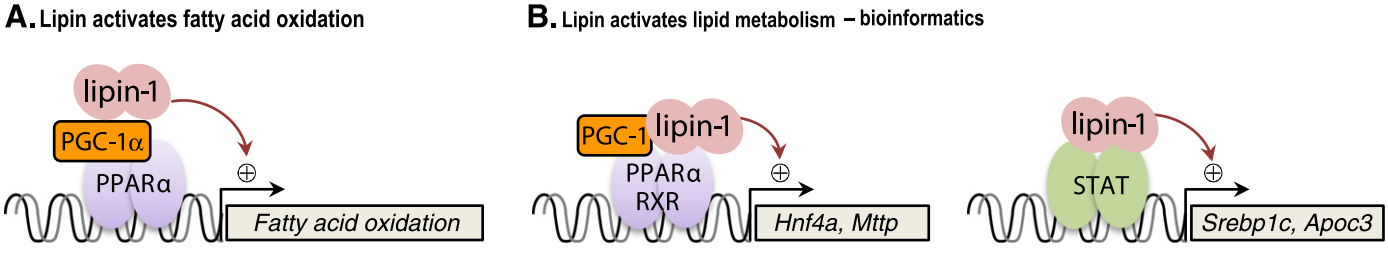

Fig. 2. Lipin and transcriptional regulation. A) Lipin-1 amplifies PGC-1α/PPARα regulatory circuits and mitochondrial fatty acid oxidation in the liver. B) Our bioinformatic analyses lead us to propose a model whereby lipin impacts transcriptional regulation of lipid metabolism genes either through interaction with PGC-1α/β or PPARα, or through interaction with other transcription factors such as STAT proteins. *Abbreviations*: *Apoc3*, apolipoprotein C-III; *Hnf4a*, hepatocyte nuclear factor-4α; *Mttp*, microsomal triglyceride transfer protein; PGC-1α/β, proliferator-activated receptor-γ (PPARγ) coactivator-1α/β; PPAR, proliferator-activated receptor-α; RXR, retinoid X receptor; *Srebp1c*, sterol regulatory element binding transcription factor-1c; STAT, signal transducer and activator of transcription.

fasting [63]. Under fasting conditions, hepatic VLDL production is stimulated as a result of reduced insulin action [64,65]. Specifically, the expression of hepatic lipin-1 is induced in response to fasting, glucocorticoids action, insulin-deficiency and diabetic conditions [25]. Glucocorticoids induce VLDL production by stimulating lipogenesis via a selective upregulation of lipin-1 expression [44], as well as by stimulating apoB translation and post-translational stability [55–57], with insulin acting antagonistically. Although the stimulatory effect of glucocorticoids on lipin-1 expression is enhanced by cAMP [44], this second messenger is reported to act as a switch to downregulate hepatic VLDL secretion [66,67]. However, the synergistic action of glucocorticoids and cAMP on hepatic lipin-1 expression, PAP activity and lipid synthesis, as well as the cAMP-induced phosphorylation of PAP [48,59] and the subsequent modulation of PAP/lipin-1 subcellular localization [23,61] may be instrumental for providing compartmentalized lipid substrates and for favoring post-translational stability of apoB [55–57], thus reversing an original and transient inhibitory effect of cAMP on VLDL secretion. While both hepatic *Lpin1* [25] and *Lpin2* [30,35] gene expression is upregulated in mice by fasting, the regulatory circuits governing their upregulation exhibit divergence. Hepatic *Lpin1* gene expression is shown to increase by PGC-1α [25]. However, PGC-1α deficiency in mice has no effect on fasting-induced *Lpin2* gene expression [35]. Likewise, PGC-1α overexpression in mouse hepatocytes also has no effect on *Lpin2* expression [35]. The lack of an influence of PGC-1α on *Lpin2* expression is reminiscent of the aforementioned glucocorticoid treatment [44] and is also observed under diabetes conditions [35]. The fasting- or glucocorticoid-induced lipin-1 expression in the liver [25] augments the potential for TAG synthesis in response to the influx of fatty acid derived from lipolysis in adipose tissue [62]. Increase in hepatic lipin-1 expression also promotes fatty acid oxidation through its transcription coactivator role in activating the expression of fatty acid oxidation genes [25].

The expression of lipin-1 is also regulated by SREBP-1 [68], the major activator of hepatic lipogenesis. A sterol regulatory element (SRE) and nuclear factor-Y binding sites reside within the human *LPIN1* promoter region, and both appear to control *LPIN1* gene transcription. Particularly, hepatic lipin1 expression is elevated in diabetic *db*/*db* mice [25], which may be attributable to elevated SREBP-1 expression/activity [69]. Although it is unclear if the increased production of hepatic DAG contributes to the insulin resistant phenotype in *db*/*db* mice, abnormal accumulation of fat in the peripheral tissues, including the liver, is reported as a major culprit [70,71]. The free fatty acid-derived accumulation of DAG is critical for the activation of PKCθ, which in turn phosphorylates insulin receptor substrate-1 and inactivates its downstream effectors [72]. Increased production of DAG via the TAG biosynthetic pathway also results in PKCε activation, which exacerbates hepatic lipid accumulation and induces insulin resistance [73]. Targeted inactivation of PKCε in nonalcoholic fatty liver disease reverses hepatic insulin resistance [74].

3.2. Post-translational modifications affect lipin compartmentalization

Lipin-1 and lipin-2 exhibit multifaceted subcellular distribution; they localize to the cytosol, microsomal membranes, and the nucleus in various cell types examined, *e.g.*, McA-RH7777, 3T3-L1, HeLa and HEK293 [23,30,35,37,75]. The compartmentalization of lipin-1, in particular, presumably underlies its dual molecular function as a glycerolipid biosynthetic enzyme and as a transactivator of lipid metabolism genes. PAP activity translocates between the cytosol and the microsomal membranes, and the membrane association potential of lipin-1 is responsible for PAP activation [48,76–78]. Translocation of lipin-1 and its encoded PAP activity between the cytosol and microsomal membranes, as well as lipin-1 partitioning between the nucleus and cytoplasm are influenced by phosphorylation status [23,61]. Lipin-1 phosphorylation is stimulated by insulin in a rapamycin-sensitive manner and results in decreased association with microsomal membranes [23,60]. In agreement with this finding, oleic acid decreases phosphorylation of lipin-1 and favors its microsomal localization [23]. The oleic acid-induced association of lipin-1 with the microsomal membranes is consistent with the known stimulatory effects of oleic acid on PAP activity and lipogenesis [76,77,79], as well as on VLDL assembly/secretion [80,81]. The effect of oleic acid on lipin translocation from cytosol to microsomal membrane is not specific to lipin-1, because binding of recombinant lipin-2 obtained from HEK-293 cells to microsomal membranes of rat liver is also enhanced by treatment with oleic acid [30]. Like lipin-1, the majority of phosphorylated lipin-2 is detected in the liver cytosol [35]. Association of lipin proteins with the microsomal membranes allows for directed localization at sites of action. Furthermore, selective phosphorylation of lipin-1 and lipin-2 by cyclin-dependent kinases [37] as well as phosphorylation of the yeast Pah1p/Smp2p [47,82] results in reduced PAP activity.

The phosphorylation sites identified in lipin-1 (Ser106, Ser634, and Ser720) are conserved in mammalian lipins and yeast Pah1p/Smp2p, of which Ser106 appears to be the major site of insulin-stimulated phosphorylation in lipin-1 [23] and lipin-2 [35]. Phosphorylation of yeast Pah1p/Smp2p is catalyzed by Cdc28p/Cdk1p [47]. The identity of a kinase(s) responsible for mammalian lipin phosphorylation remains to be determined. Dephosphorylation of the yeast Pah1p/Smp2p is catalyzed by a complex consisting of Nem1p and Spo7p [47]. The mammalian counterpart that dephosphorylates lipin-1 is Dullard [83]. Dephosphorylation of Pah1p/Smp2p by the Nem1p-Spo7p complex at the ER/nuclear membrane results in higher PAP activity, lower PA levels, and translocation of Opi1p into the nucleus to repress transcription of phospholipid synthesis genes [29,82]. Thus, phosphorylation of lipin proteins strongly influences their function through altered subcellular localization and through transcriptional regulation of phospholipid synthesis genes.

Lipin-1α and lipin-1β undergo sumoylation on two consensus sumoylation sites (Fig. 1) in cell culture models and in rodent brain. Although lipin-2 contains one sumoylation consensus site (Fig. 1), no sumoylation of lipin-2 is observed in transfected HeLa cells [42]. The potential sumoylation site of lipin-2 may be inaccessible in its three-dimensional structure or sumoylation of lipin-2 may occur in a cell-specific perspective. Consensus sumoylation sites are not present in mammalian lipin-3 or orthologs in yeast or invertebrates [42]. Sumoylation of lipin-1α stimulates its nuclear localization in both embryonic cortical neurons and cultured SH-SY5Y neuronal cells, and mutation of sumoylation sites within lipin-1α hampers its nuclear localization and its ability to coactivate the transcriptional coactivators PGC-1α and MEF2 [42]. Sumoylation may facilitate nuclear localization of lipin-1α in neuronal cells by controlling interactions with the nuclear import/export machinery, and/or by regulating its binding to intranuclear components. Sumoylation sites are located in close proximity to nuclear export signals [84]. Thus, sumoylation of lipin-1α might affect its export from nucleus by interfering the binding of nuclear export signals with their receptors. The functional significance of lipin-1β sumoylation is unknown.

3.3. Nuclear localization signal sequences affect lipin compartmentalization

All lipin proteins contain one or more nuclear localization signals (NLS) (Fig. 1), which allow access to the nucleus and interactions with DNA-bound transcription factors. In the fission [85] and budding [29,47] yeast, as well as in the nematodes [86,87], the corresponding lipin orthologs all locate to the nucleus. In particular, Pah1p/Smp2p associates with the nuclear membrane and chromatin in *S. cerevisiae* [47], and the lipin homolog in *C. elegans* localizes to the nucleoplasm, ER and nuclear envelope [86]. Deletion of the NLS sequences [8] completely excludes lipin-1α from the nucleus in 3T3-L1 adipocytes [61] and in hepatocytes [75]. However, although both lipin-1α and lipin-1β contain NLS sequences, lipin-1α is localized primarily in the

nucleus and lipin-1β is present in cytoplasm. This unique distribution between lipin-1α and lipin-1β has been observed in pre- and post-differentiated adipocytes [14,61], rat hepatoma McA-RH7777 cells [75], and embryonic cortical neurons and cultured SH-SY5Y neuronal cells [42]. Apparently, the NLS sequences do not solely encode lipin-1 nuclear localization.

The functional significance of nuclear localization of lipin-1α in hepatic VLDL assembly/secretion has been tested by mutagenesis studies. Deletion of the NLS sequences in lipin-1α abolishes its nuclear localization, but has no effect on the encoded PAP activity [75]. The NLS deletion (ΔNLS) mutant form of lipin-1α not only loses its nuclear localization but also the ability to associate with microsomal membranes [75], suggesting that the NLS sequences may play a role in membrane targeting. Expression of the ΔNLS mutant of lipin-1α results in lowered synthesis and secretion of TAG and apoB-100, as compared with the wild-type lipin-1α [75]. These cell culture studies provide evidence that subcellular localization of lipin-1α impacts to a huge extent hepatic lipogenesis and VLDL secretion.

3.4. Protein–protein interactions affect lipin compartmentalization

Additional molecular mechanisms that regulate lipin-1 nucleo-cytoplasmic distribution have been identified. For instance, sumoylation is critical for nuclear localization of lipin-1α in neuronal cells [42], and interaction of lipin-1α with 14-3-3 proteins is a determinant for its cytoplasmic localization in HEK293 cells and 3T3-L1 adipocytes [61]. In particular, expression of 14-3-3 β and θ isoforms in adipocytes promotes cytoplasmic over nuclear localization of lipin-1α. The interaction with 14-3-3 proteins and cytoplasmic localization of lipin-1α in 3T3-L1 adipocytes are promoted by insulin, which increases lipin-1 phosphorylation [61].

Post-translational modifications and protein interaction networks of lipin proteins play a critical role in enzymatic activation for glycerolipid biosynthesis and transcriptional regulation of lipid metabolism genes through regulating lipin microsome-cytosol compartmentalization and nucleo-cytoplasmic shuttling (Fig. 3).

4. The dual function of lipin impacts several aspects of VLDL assembly/secretion

4.1. Lipid substrates (cargo) for VLDL assembly/secretion are regulated by lipin-1

The TAG-rich VLDL particles are assembled within the ER/Golgi lumen, a process requires active biosynthesis of the lipid constituents (*e.g.*, phospholipids and TAG) and partitioning of TAG into the ER/Golgi lumen [2,88]. Clinically, impaired secretion of hepatic VLDL is frequently associated with massive accumulation of TAG in the liver (termed hepatosteatosis), which is common in nonalcoholic fatty liver diseases. Hepatic TAG synthesis occurs at the ER, and the resulting TAG is present both in the cytosol (as a storage pool in the form of lipid droplets) and in association with ER/Golgi [89,90]. The cytosolic TAG pool is metabolically connected to the ER/Golgi reservoir via a process termed "hydrolysis/reesterification" through which the storage TAG is utilized as substrate for VLDL assembly [91,92]. The activity of MTP is required for accumulation of TAG within the ER/Golgi lumen and thus essential for VLDL assembly/secretion [80,93,94]. In addition to hydrolysis/reesterification of TAG, hydrolysis of phospholipids also contributes fatty acyl substrates for TAG synthesis during VLDL assembly/secretion [95–97]. The process of phospholipid-hydrolysis/TAG-reesterification in hepatic cells may be compartmentalized, and fatty acyl chains derived from PC or PE (*e.g.*, 18:1(n−9) and 20:5(n−3)) are utilized differently for VLDL assembly/secretion [81].

From the VLDL lipid substrate (cargo) point of view, the reaction catalyzed by lipin provides key glycerolipids (*e.g.*, TAG, PC, and PE) that are synthesized either directly from the *de novo* pathway [27,62] or indirectly from the hydrolysis/reesterification pathway [81,92,98].

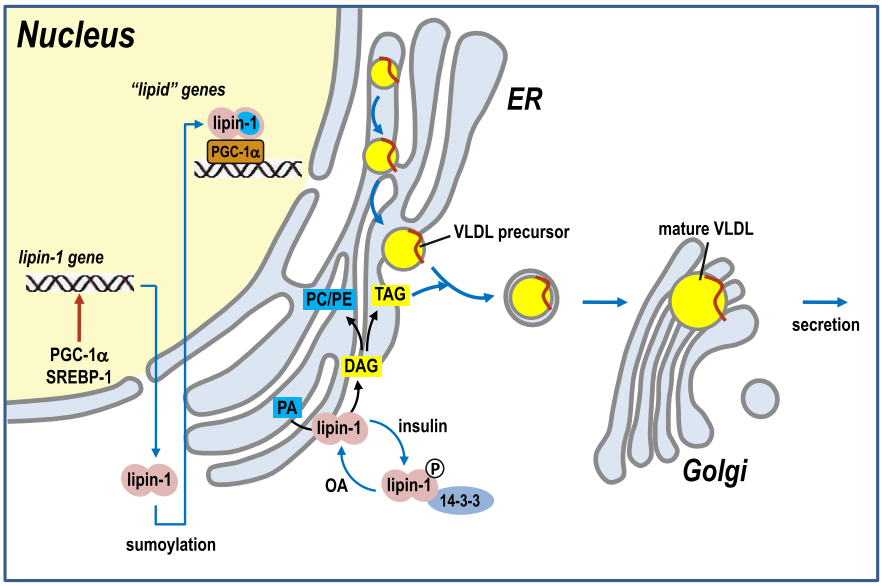

Fig. 3. A model depicting the effect of lipin-1 gene expression, post-translational modifications and its protein interaction networks on PAP enzymatic activity and transcriptional regulation of lipid metabolism — consequences on VLDL assembly and ER/Golgi membrane dynamics. Transcriptional regulators of lipid homeostasis are involved in establishing or maintaining lipin-1 gene expression. SREBP-1 stimulates human *LPIN1* [68] and mouse *Lpin1* [25,69,157] gene expression, and PGC-1α activates hepatic lipin-1 expression, which is also upregulated during fasting in mice (26) and by glucocorticoids [44]. Insulin stimulates phosphorylation of lipin-1, which favors its interaction with 14-3-3 proteins and thus cytoplasmic over nuclear localization of lipin-1. Nucleo-cytoplasmic shuttling of lipin-1 is also regulated by sumoylation, and a phosphatase complex located at the ER/nuclear membrane stimulates the interaction of the lipin-1 ortholog in yeast with phospholipid biosynthetic genes and ER/nuclear membrane biogenesis [47]. Phosphorylation also controls cytosol-microsome compartmentalization of lipin-1, with oleic acid (OA) promoting lipin-1 association with the ER membrane and its subsequent activation as a glycerolipid synthetic enzyme. The lipin-1 induced PAP reaction generates lipid substrates (TAG, PC, and PE) required for lipidation of the nascent apoB particle in the ER lumen to generate VLDL precursors and for VLDL maturation in post-ER subcompartments (lipid *cargo* function of lipin-1), as well as for ER/Golgi membrane dynamics (lipid *conveyor* function of lipin-1) and transport vesicle biogenesis during VLDL trafficking and secretion. *Abbreviations*: SREBP-1, sterol regulatory element binding protein-1; PGC-1α, peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α.

Recent transient transfection experiments with hepatic cell cultures have shown that the expression of either the nucleus-bound lipin-1α or the cytoplasm-bound lipin-1β results in increased synthesis and secretion of TAG-rich VLDL [75]. Conversely, knockdown of lipin-1 in these cells decreases the secretion of TAG even though the total cellular PAP activity is unaffected (presumably owing to the presence of lipin-2 and lipin-3) [75]. These cell culture studies indicate that, although hepatic lipin-1 plays an important role in TAG synthesis and VLDL secretion, the presence of hepatic lipin-2 and lipin-3 contribute to a complex regulatory mechanism. This may explain why hepatic PAP activity is unchanged [24] or moderately decreased [23] in adult *fld* mice, and why neonatal *fld* mouse liver exhibits increased PAP activity despite the lack of lipin-1 expression [35,41]. Determination of glycerolipid species in steatotic neonatal *fld* liver by electrospray ionization-mass spectrometry (ESI-MS) has shown a decrease in PA that is accompanied by a drastic increase in TAG but not PC, PE or phosphatidylserine (PS) [35], indicating that the lack of lipin-1 may be compensated by lipin-2 and/or lipin-3 for normal PAP activity. Independent of changes in steady-state *Lpin2* mRNA levels in the liver of neonatal or adult *fld* mice, the lipin-2 protein concentration is drastically increased due to accelerated rates of translation [35]. Overexpression of lipin-2 in HepG2 cells, which express little endogenous lipin-2, yields robust PAP activity, whereas knockdown of lipin-2 in normal or *fld* hepatocytes decreases PAP activity and TAG synthesis in spite of a compensatory increase in *Lpin3* mRNA [35]. These results suggest that lipin-2 can compensate for lipin-1 deficiency in *fld* hepatocytes. However, the observed high protein expression of lipin-2 [24,30] and upregulated expression of *Lpin3* mRNA [24] in *fld* hepatocytes have not been uniformly verified by other laboratories [41].

The dual functionality of lipin-1 and multiplicity of lipin isoforms make it challenging to determine the cellular or molecular actions of lipin-1 in promoting or attenuating hepatic VLDL secretion. While cell culture studies suggest that expression of lipin-1 promotes TAG biosynthesis in hepatic [75] and cardiac myocytes [99], and enhances hepatic VLDL assembly/secretion [75], studies with the *fld* mouse model indicate that lipin-1 deficiency increases plasma VLDL levels [25]. Moreover, adenoviral-mediated overexpression of lipin-1β in *fld* hepatocytes results in decreased VLDL secretion [41]. The hypertriglyceridemia in *fld* mice during the suckling period may not be attributable to overproduction of hepatic VLDL but rather to lower lipoprotein lipase and hepatic lipase activities [5]. Owing to the transcriptional coactivator activity of lipin-1, adenovirus-mediated overexpression of lipin-1 may suppress the expression of gene products that promote VLDL assembly/secretion, such as *Scd1*, *Apoa4* and *Apoc3* [41]. The *Scd1* gene encodes steroyl-CoA desaturase whose expression correlates positively to plasma TAG concentrations in humans and mice [100,101]. The *Apoa4*/*Apoc3*/*Apoa1* gene locus is associated with atherosclerosis [102,103]. Variations in *Apoa4* are also associated with altered plasma TAG levels [104], and expression of apoA-IV in intestinal cells promotes TAG-rich lipoprotein secretion [105,106]. Deficiency in *APOC3* in humans leads to low plasma TAG concentrations and reduced incidence of coronary artery calcification [107]. Expression of apoC-III in hepatic cells stimulates the assembly and secretion of TAG-rich VLDL under lipid-rich conditions [108]. The interplay of various VLDL lipid and protein factors whose expression and availability are influenced by lipin-1 in the liver requires further exploring.

4.2. Membrane dynamics (conveyor) for VLDL assembly/secretion are regulated by lipin-1

The assembly and secretion of VLDL involves intricate intracellular ER/Golgi membrane fission and fusion events. There is a debate in the literature about the location and mode of assembly of VLDL particles. Several studies demonstrate that VLDL assembly is completed in the ER before the particle enters the Golgi apparatus in primary hepatocytes and in McA-RH7777 cells [109–111]. However, experimental evidence also suggests that VLDL maturation may occur in post-ER or in the Golgi compartment [112–115]. It is suggested that hepatic and intestinal cells have evolved post-ER subcompartments that are dedicated to the maturation of apoB and lipoprotein particles [115]. Reconstitution of vesicle budding using microsomes isolated from rat hepatoma McA-RH7777 cells reveals that vesicles containing apoB-100 are distinct from 'typical' protein/cargo trafficking vesicles [112,115]. It is also shown that pre-chylomicron transport vesicles targeted from the ER to the Golgi apparatus harbor newly synthesized TAG, apoB-48, MTP, lipid related proteins and vesicular transport proteins in enterocytes [116–118]. This is indicative of the importance of these transport vesicles for formation, transport, lipidation, and assembly of chylomicron particles. Various isoforms of PKC participate in the formation of Golgi vesicles and vesicular tubular clusters [119,120]. In particular, PKCζ-mediated phosphorylation controls budding of the pre-chylomicron transport vesicles [121]. PKCζ [122] requires PA for activation [123], and DAG is essential for protein trafficking from the Golgi complex in yeast [124]. This indicates that the lipin-driven PAP reaction and the balance between substrate (PA) and product (DAG) may be a prerequisite for vesicle and lipoprotein trafficking across the cell.

From a lipoprotein assembly and intracellular trafficking point of view, glycerolipid biosynthesis not only governs the VLDL assembly process but also the function and dynamics of ER/Golgi membranes (conveyor). The lipin-encoded PAP activity and transcriptional regulator function in phospholipid biosynthesis are crucial for glycerolipid metabolism, membrane trafficking dynamics and lipoprotein maturation events. Lipin may coordinate membrane synthesis and provide lipid substrate during vesicular trafficking of hepatic apoB100 and its maturation to VLDL particles. Specifically, lipin may orchestrate the synthesis of the phospholipid coat of budding vesicles containing large biological assemblies/lipoproteins or cargos (transport vesicle biogenesis) destined to exit the ER en route for secretion (Fig. 3). Mammalian lipin-1 stimulates phospholipid synthesis and ER membrane expansion in B lymphocytes undergoing differentiation [125]. Inactivation of LPIN-1 in *C. elegans* leads to ER disorganization and defective nuclear envelope breakdown [86,87]. In *S. cerevisiae*, deficiency of Pah1p/Smp2p or its dephosphorylated form leads to a massive expansion of the nuclear envelope and abnormal nuclear/ER membrane structure due to transcriptional upregulation of key enzymes involved in phospholipid biosynthesis, as well as due to the lack of PAP activity [29,47]. Moreover, the ortholog of lipin-1 in *S. pombe* (Ned1p) interacts with three nuclear proteins that are important for nuclear envelope formation [85], suggesting further the importance of lipin and its interaction networks in nuclear morphology and biogenesis.

Phospholipid biosynthesis in yeast is transcriptionally induced in response to the need for more ER membrane during the unfolded protein response [126], indicating that lipid metabolism is coordinated with ER growth. In mammals, enforced expression of XBP1, which is activated during the unfolded protein response, increases the activity of enzymes involved in PC biosynthesis and causes expansion of the ER [127]. Mutations in the lipin gene family may result in aberrant ER structures, which may disrupt vesicular transport [128]. The functional link between lipin-mediated lipid biosynthesis and ER/nuclear membrane dynamics in the context of VLDL assembly/secretion requires further investigation.

5. Conclusion and Perspectives

Identification of the originally characterized nuclear protein lipin-1 as the PAP enzyme has created a remarkable opportunity to gain insights into regulation of lipid metabolism at transcription and enzymatic levels. The dual function of lipin-1 in catalyzing the key reaction of the biosynthesis pathway for glycerolipids and in acting as transcriptional regulators has placed it in a key position in regulating hepatic lipid/lipoprotein metabolism. The multiplicity of lipin isoforms and their intricate compensatory regulation as exemplified in the *fld*

mouse model further highlight the functional significance of lipin-encoded PAP activity and transcriptional regulatory circuits in maintaining cellular DAG homeostasis and also the lack of functional redundancy amongst lipin isoforms. Post-translational modifications and associated lipin compartmentalization, particularly the nuclear and microsomal localization, appear to govern the dual function of lipin proteins. What remains to be unraveled is the target genes that are influenced by different lipin isoforms in cooperation with other known and unknown transcription factors involved in lipid metabolism. Of particular interest is to gain a comprehensive view, through systems biology approach, on the post-translational modifications and protein interaction networks of lipin under various metabolic conditions, such as lipotoxicity-induced ER stress, insulin resistance and diabetes, and to determine the consequences on lipin function in glycerolipid synthesis, ER/nuclear membrane biogenesis and lipoprotein metabolism and trafficking.

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