Three Mammalian Lipins Act as Phosphatidate Phosphatases with Distinct Tissue Expression Patterns*

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We previously identified mutations in the Lpin1 gene, encoding lipin-1, as the underlying cause of lipodystrophy in the fatty liver dystrophy (fld) mutant mouse. Lipin-1 is normally expressed at high levels in adipose tissue and skeletal muscle, and deficiency in the fld mouse causes impaired adipose tissue development, insulin resistance, and altered energy expenditure. We also identified two additional lipin protein family members of unknown function, lipin-2 and lipin-3. Han et al. (Han, G. S., Wu, W. I., and Carman, G. M. (2006) J. Biol. Chem. 281, 9210–9218) recently demonstrated that the single lipin homolog in yeast, Smp2, exhibits phosphatidate phosphatase type-1 (PAP1) activity, which has a key role in glycerolipid synthesis. Here we demonstrate that lipin-1 accounts for all of the PAP1 activity in white and brown adipose tissue and skeletal muscle. However, livers of lipin-1-deficient mice exhibited normal PAP1 activity, indicating that other members of the lipin protein family could have PAP1 activity. Consistent with this possibility, recombinant lipin-2 and lipin-3 possess PAP1 activity. Each of the three lipin family members showed Mg²⁺-dependent activity that was specific for phosphatidate under the conditions employed. The different lipins showed distinct tissue expression patterns. Our results establish the three mammalian lipin proteins as PAP1 enzymes and explain the biochemical basis for lipodystrophy in the lipin-1-deficient fld mouse.

Triacylglycerol (TAG)³ plays a key role in metabolic homeostasis, serving as the major energy storage molecule that allows organisms to survive periods of food deprivation. The regulation of TAG storage is important in human disease because both excessive and inadequate fat storage is associated with dyslipidemia, insulin resistance, and diabetes (reviewed in Refs. 1–3). We previously characterized the fatty liver dystrophy mouse, a model of generalized lipodystrophy with impaired TAG storage in adipose tissue, insulin resistance, and increased susceptibility to atherosclerosis (4, 5). Lipodystrophy in the fld mouse results from mutation in the Lpin1 (lipin-1) gene, the founding member of a family of three genes of previously unknown function (6). Genes for lipin-1, lipin-2, and lipin-3 occur in mammals and other vertebrates, whereas a single lipin gene ortholog can be detected in evolutionarily distant organisms including fruit fly, nematode, plants, and yeast (6). This suggests a fundamental function for lipin that is conserved from single celled eukaryotes to mammals.

In the mouse, lipin-1 is expressed at high levels in adipose tissue and skeletal muscle, consistent with a role in lipid metabolism in these tissues. Indeed, adipocytes in lipin-1-deficient mice fail to accumulate TAG and do not develop mature adipocytes (7). By contrast, transgenic mice with enhanced lipin-1 expression in adipocytes accumulate more TAG per cell and are prone to obesity (7–9). Furthermore, lipin-1 expression levels are reduced in adipose tissue of human lipodystrophic patients concomitantly with reduced fat mass (10). A role for lipin-1 in muscle metabolism is suggested by increased energy expenditure and fatty acid oxidation in the muscle of lipin-1-deficient mice and the opposite effects in muscle-specific lipin-1 transgenic mice (8). Thus, alterations in lipin-1 expression levels in either adipose tissue or skeletal muscle produce important physiological effects on energy storage and expenditure.

In mammalian cells, the de novo biosynthesis of TAG, PC, and phosphatidylethanolamine is catalyzed mainly through the glycerol phosphate pathway (11). Several enzymes in this pathway have been characterized, but not all of these have been identified at the molecular level. Among those for which a gene has not been isolated is the phosphatidate phosphohydrolase (phosphatase) type-1 that converts the PA formed from glycerol phosphate and lysoPA to DAG (12). There are two main types of PA phosphatase. The first is the type-1 activity (PAP1) that is characterized by its inhibition by N-ethylmaleimide (NEM) and a complete dependence on Mg²⁺ (12, 13). In contrast, the second activity (PAP2) is neither inhibited by NEM nor is it stimulated by Mg²⁺. There are three PAP2 enzymes that are integral membrane proteins (14). They catalyze the
Mammalian Lipins Are Phosphatidate Phosphatases

hydrolysis of a variety of lipid phosphate esters including lysoPA, C1P, S1P, and DAG pyrophosphate in addition to PA. Because of this and the uncertainty of the physiological substrates for these enzymes, the three PAP2 enzymes were renamed lipid phosphate phosphatases (LPPs) (15). These enzymes control signal transduction by the bioactive lipid phosphates compared with their dephosphorylated products (14).

The phenotype of the lipin-1-deficient fld mouse is consistent with a defect in TAG synthesis, raising the possibility that lipin-1 may perform a role in glycerolipid biosynthesis. Recently, Han et al. (16) reported a breakthrough in the identification of the Mg$^{2+}$-dependent PAP1 activity from Saccharomyces cerevisiae. Through protein sequencing, the yeast PAP1 is shown to be identical to Smp2, the yeast ortholog of mammalian lipin. Recombinant human lipin-1 expressed in Escherichia coli also shows PAP1 activity (16). However, the extrapolation of studies on yeast lipin to its role in mammals is complicated by the fact that mammals possess three lipin genes. Furthermore, alternative mRNA splicing of the mouse and human lipin-1 gene gives rise to two protein isoforms, lipin-1A and lipin-1B, which have distinct properties in adipocytes (9). Specifically, the addition of 33 internal amino acids to the lipin-1B isoform leads to different expression dynamics during adipocyte differentiation, altered subcellular localization, and functional differences in induction of gene expression during adipocyte differentiation (5, 9).

Here we utilize the fld mouse to establish the physiological role of lipin-1 as the sole PAP1 enzyme in adipose tissue and muscle, largely explaining the biochemical basis for lipodystrophy in these mice. We also determine that all known mammalian lipin family members and isoforms exhibit PAP1 activity that is specific for phosphatidate. The different lipins exhibit distinct tissue expression patterns, suggesting unique physiological roles for each in glycerolipid synthesis.

EXPERIMENTAL PROCEDURES

Animals—C57BL/6J and BALB/cByl-Lpin1$^{+/fld}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The latter were bred to generate homozygous mutant (fld/fld) and wild-type (+/+). Animals were fed Purina 5001 mouse chow and were maintained on a 12:12 h light:dark cycle. Tissues were collected in the first 2 h of the light cycle. Animal studies were performed under approval of the UCLA animal care committee.

Preparation of Labeled Lipid Phosphates—PA labeled with [3H]palmitate was prepared as described by Martin et al. (17). S1P plus lysoPA and C1P were labeled with $^{32}$P using [$γ$-$^{32}$P]ATP and homogenates from Rat2 fibroblasts that overexpressed sphingosine kinase-1 and DAG kinase from E. coli, respectively. The labeled lipids were extracted and purified as described previously (18). These lipids were then added to preparations of the equivalent unlabeled lipids to obtain the desired specific radioactivity.

RNA Quantitation by Real-time RT-PCR—A mouse tissue expression panel was produced from tissues of three C57BL/6J mice. Total RNA was isolated with TRIzol (Invitrogen) and cDNA synthesized from 1 µg of RNA using Omniscript reverse transcriptase kit (Qiagen, Valencia, CA). Real-time RT-PCR was performed with the iCycler (Bio-Rad) using SYBR Green PCR reagents (Qiagen). Gene expression was normalized to housekeeping genes, hypoxanthine phosphoribosyltransferase and β-2 microglobulin. Expression in human tissues was performed using panels of normalized, first strand cDNA preparations (Human MTC Panel I and Digestive System MTC Panel) and from a pooled human adipose tissue sample (Clontech). Primer sequences are provided in supplemental Table S1.

Lipin Expression Constructs—Expression vectors for lipin-1A, lipin-1B, lipin-2, and Lipin-3 were generated by PCR amplification of the coding region and subcloning into the pcDNA 3.1/V5-His expression vector (Invitrogen) between the EcoRI/NotI restriction enzyme sites for lipin-1A, lipin-1B, and lipin-3 and the EcoRV/NotI restriction enzyme sites for lipin-2. Sequences for lipin cDNAs were as follows: lipin-1A, GenBank accession no. NM_172950; lipin-1B, NM_015763; lipin-2, NM_022882; lipin-3, NM_022883. All expression constructs were verified by sequencing. Primers used for PCR amplification are provided in supplemental Table S2.

Cell Culture and Transfection—293T cells (American Type Culture Collection, Manassas, VA) were propagated in Dulbecco’s modified Eagle medium containing 10% fetal bovine serum and supplemented with antibiotics at 37 °C and 5% CO2. Cells were transfected with Effectene transfection reagent (Qiagen). Two days after transfection, the cells were lysed and assayed for PAP1 activity as described below.

Measurement of PAP1 and PAP2 (LPP) Activities—Mouse tissues were homogenized in 0.25 m sucrose containing 2 mM dithiothreitol (to stabilize PAP1 activity), protease inhibitor mixture (EDTA-free, Roche Diagnostics), and phosphatase inhibitor cocktails I and II (Sigma-Aldrich). 293T cells were harvested in the same medium to which 0.15% Tween 20 was added to lyse the cells and internal membranes and ensure complete release of PAP1 activity. We showed previously that Tween 20 stabilizes and stimulates PAP1 activity (19). We designed an optimum assay (0.1 ml final volume) for PAP1 using 100 mM Tris/maleate buffer, pH 6.5, 5 mM MgCl2, 2 mg/ml fatty acid-poor bovine serum albumin and 0.6 mM PA labeled with [3H]palmitate (about 1 × 10^6 dpm/assay), which was dispersed in 0.4 mM PC and 1 mM EDTA that was used to prepare the lipid substrate (13, 17). When Tween 20 was added to the cell extracts, its final concentration in the assay was adjusted to 0.05%. Reactions were stopped after incubation at 37 °C with 2.2 ml of chloroform containing 0.08% olive oil as carrier for neutral lipids. Then 0.8 g of basic alumina was added to adsorb the PA and any [3H]palmitate formed by phospholipase A type activities (17). The tubes were centrifuged and 1 ml of chloroform, which contained the [3H]DAG product, was dried and quantitated by scintillation counting. Protein concentrations (measured by the Bradford method) and the times of incubation (normally 30 min) were adjusted so that <15% of the PA was consumed during the incubation. Total PAP activities were calculated from measurements at three different protein concentrations to ensure the proportionality of the assay. The dilution of the samples in the assay was adjusted to ensure that vanadate concentrations from the phosphatase inhibitor mixture were <500 µM so as not to inhibit PAP1 activity.
Mammalian Lipins Are Phosphatidate Phosphatases

Parallel incubations were performed in the presence of excess (5 mM) NEM to inhibit PAP1 and to compensate for any PAP2 (LPP) activity in this assay. This latter value was normally <10% of the total activity from which it was subtracted to give true PAP1 activity.

More detailed kinetic analysis of the PAP1 activities for the different lipins was obtained using a surface dilution kinetic model essentially as described by Han et al. (16). For this, we used lysates from the 293 cells that were diluted such that the final concentration of Tween 20 present in the assays was 1.22 μM. Each assay contained in a final volume of 0.1 ml: 50 mM Tris/HCl (pH 7.4), 1 mM MgCl2, 2 mM Triton X-100, 2 mg/ml fatty acid poor bovine serum albumin, and the appropriate concentrations of PA containing about 1.2 × 106 dpm. DAG formation was measured as described above; values obtained in the presence of 5 mM NEM were subtracted from those in its absence to obtain PAP1 activity. The level of PAP1 measured for the untransfected cells was negligible under these conditions such that the PAP1 reflected that of the overexpressed lipins. NEM-inhibited phosphatase activity against 8 mol % of PA was also compared against that obtained with equivalent concentrations of [32P]-labeled lysoPA, S1P, or C1P (about 1 × 106 dpm/assay). Phosphatase activity was measured after the extraction of the inorganic [32P]phosphate as described previously (20). Kinetic analysis of the PAP1 activities was performed using the EZ-FIT enzyme model fitting program (21).

LPP activities were measured in the absence of a contribution from PAP1 by using assays with [3H]PA in micelles in Triton X-100 (20). The incubations contained no PC or Mg2+, and excess (5 mM) NEM was added. The proportionality of the assay and purification and quantitation of [3H]DAG were achieved as described for PAP1.

Western Blot Analysis—Protein concentrations in cell lysates were determined using the Bio-Rad protein assay. Similar amounts of protein were electrophoresed in 6% SDS-polyacrylamide gels (Invitrogen) and transferred to nitrocellulose membranes using a semi-dry electrophoret (Bio-Rad). Membranes were blocked with 5% nonfat milk powder in Tris-buffered saline and incubated overnight with mouse monoclonal anti-V5 antibody (Invitrogen). Membranes were then incubated for 1 h at room temperature with a 1:10,000 dilution of goat anti-mouse IgG conjugated to Alexa Fluor 680 (Molecular Probes, Eugene, OR). Fluorescence at 700 nm was detected with the Odyssey infrared imaging system and quantified with Odyssey software. Mouse tissue Western blots were prepared from wild-type and fld tissue extracts and detected with a primary rabbit anti-lipin antibody (raised against the peptide SKTDSPSRKDKRSRHLGADG) followed by goat anti-rabbit IgG secondary antibody and developed with enhanced chemiluminescence reagents (GE Healthcare).

RESULTS

Mammalian Lipins Possess the DXDXT Motif Characteristic of Mg2+-dependent Phosphatases—The yeast PAH1 protein sequence contains a haloacid dehalogenase domain, which includes a DXDXT motif found in a superfamily of Mg2+-dependent phosphatases (16, 22). This motif is also present in lipin proteins from other species, including mouse and human (data not shown). It occurs within the previously defined C-LIP domain (6) and has the invariant sequence DIDGT in lipin-1 orthologs found in mammals, chicken, fish, Drosophila, Ciona, and S. cerevisiae (www.ensembl.org). The DIDGT motif is also present in mammalian lipin-2 and -3 (data not shown) suggesting that all lipin proteins may exhibit PAP1 activity.

Lipin-1 Is Responsible for PAP1 Activity in Adipose Tissue and Skeletal Muscle—The impaired TAG storage in adipose tissue and muscle of lipin-1-deficient fld mice suggested that lipin-1 could be the critical PAP1 enzyme in these tissues. To investigate this, we assayed several tissues from fld mice for PAP1 activity. As a control, we also determined the LPP activity, which is catalyzed by three enzymes that are unrelated in structure to the lipins. As shown in Fig. 1A, no significant PAP1 activity was detected in gonadal and subcutaneous white adipose tissue, in intrascapular brown adipose tissue; BAT, interscapular brown adipose tissue; *, different from corresponding wt tissue at p < 0.005; **, p < 0.001.

FIGURE 1. Absence of PAP1 activity in adipose tissue and muscle of lipin-deficient fld mice. Tissues prepared from fld and wild-type (wt) littersmates were assayed for (A) Mg2+-dependent PAP1 activity and (B) Mg2+-independent LPP1 activity. Activity is normalized to protein content of tissue extracts and represents the mean values ± S.D. for tissues from three mice of each genotype. Ing WAT, gonadal white adipose tissue; InS WAT, inguinal subcutaneous white adipose tissue; BAT, interscapular brown adipose tissue. **, different from corresponding wt tissue at p < 0.005; *, p < 0.001.

As described above, the specific activity of PAP1 in the liver of wild-type mice was much lower than that in adipose tissue. Consistent with the lower PAP1 activity levels, lipin-1 protein levels in wild-type liver were also much lower than those in...
Mammalian Lipins Are Phosphatidate Phosphatases

Adipose tissue (Fig. 2A). As expected, no lipin-1 protein was detected in tissues from the fld mouse; however, as shown in Fig. 1A, PAP1 activity in fld liver is comparable with wild-type. This raised the possibility that lipin-2 and/or lipin-3 may compensate for the absence of lipin-1 in fld liver. To investigate this possibility, we quantitated lipin-2 and lipin-3 mRNA levels in wild-type and fld liver. We found that both genes are expressed in liver, and that lipin-3 expression is increased 4-fold in wild-type and fld mice determined by real-time RT-PCR. *p < 0.005 versus wild-type.

Lipin-2 and Lipin-3 Exhibit PAP1 Activity—The conservation of the Mg\(^{2+}\)-dependent phosphatase motif in lipin-2 and lipin-3 suggested that these family members also have PAP1 activity. In addition, the existence of two lipin-1 protein isoforms derived from alternative mRNA splicing (lipin-1A and lipin-1B) raised the question of whether both lipin-1 isoforms exhibited PAP1 activity. The 33 amino acid difference between lipin-1A and lipin-1B was consistent with the development of the C terminus of each recombinant protein (Fig. 3B). We found that both lipin-1 isoforms exhibited PAP1 activity (Fig. 3B). However, the B isoform consistently exhibited about 1.7-fold higher PAP1 activity when normalized to the V5 epitope. Lipin-2 and lipin-3 also had PAP1 activity, although at about one-fourth the relative specific activity of lipin-1A. These results were obtained under conditions where PAP1 activity was proportional to the amount of each lipin added to the assays, and they establish that all mammalian lipins have PAP1 activity.

To investigate further the kinetics of the PAP1 activities of the three lipin proteins, we employed a surface dilution kinetic model using micelles of Triton X-100 (16). Under the conditions used, the activity of PAP1 in the control 293 cells was negligible, and all PAP1 activity was accounted for by the overexpressed lipin. The relative V\(_{\text{max}}\) values calculated for the PAP1 activities of the lipins again showed that the activity for lipin-1B was greater than lipin-1A (Fig. 4A, Table 1). In this Triton X-100 micelle assay, the calculated V\(_{\text{max}}\) for the PAP1 activity of lipin-2 was similar to that for lipin-1A, with lipin-3 showing lower relative activity. Each lipin exhibited a strong positive cooperativity for PA as demonstrated by the apparent Hill coefficient (Table 1). The apparent K\(_{\text{app}}\) values for the four lipins ranged from 7 to 9 mol %. Each of the lipins showed strong Mg\(^{2+}\)-dependence (Fig. 4, B and C). These results confirm that all of the mammalian lipins possess PAP1 activity. However, the measurements were made with cell homogenates, and we cannot be certain that the kinetic constants...
We also tested lysoPA, C1P, and S1P as substrates for the different lipins. Under the conditions used to study PA hydrolysis in the same experiment, we observed no significant Mg\textsuperscript{2+}-dependent phosphatase activity with C1P, S1P, and lysoPA for any of the overexpressed lipins (results not shown).

**Mouse and Human Lipin-1, -2, and -3 Genes Exhibit Distinct Tissue expression Patterns**—Real-time RT-PCR analysis revealed that the three lipin family members exhibit unique, but overlapping, tissue distributions. Mouse lipin-1 was most prominent in skeletal muscle, with lower levels in adipose tissue depots, brain, and liver (Fig. 5A). These results differ from our previous observation that lipin-1 is expressed at equally high levels in mouse adipose tissue and muscle (6) and may reflect a strain difference (C57BL/6J here versus BALB/cByJ in previous studies). In humans, lipin-1 mRNA was most abundant in adipose tissue followed by skeletal muscle, with lower expression detected in some portions of the digestive tract (Fig. 5B). Mouse and human lipin-2 were expressed at substantial levels in liver and brain, with human lipin-2 also exhibiting unexpectedly high expression in adipose tissue (Fig. 5, C and D). Lipin-3 showed a very distinct pattern, with significant expression primarily in intestine and other regions of the gastrointestinal tract (Fig. 5, E and F). Thus, the tissue patterns observed in mouse and human are similar, aside from the high expression of lipin-2 detected in human, but not mouse, adipose tissue. The basis for this discrepancy may be a true biological difference. Alternatively, it may represent a peculiarity of the human pooled adipose tissue sample that was available. This came from unknown donors and unspecified adipose tissue depots. It should also be emphasized that different primer sets were used for the different lipins. Therefore, the relative expression values for mRNA can be compared for each lipin but not among the lipins. Overall, these results suggest that each of the lipin family members may perform similar biochemical functions but may act in a tissue-specific manner.

**DISCUSSION**

Our results demonstrate for the first time that all three members of the mammalian lipin protein family exhibit NEM-inhibitable PAP1 activity. The lipin activities were specific for PA, and they depended upon Mg\textsuperscript{2+}. The lipins exhibit strong cooperativity toward PA concentrations, which resembles the results of Han et al. (16) for yeast PAP1 (Pah1p) activity. No significant phosphatase activity was detected under the conditions used for the mammalian lipins against lysoPA, C1P, and S1P. Our results demonstrate that the mammalian lipins are distinct Mg\textsuperscript{2+}-dependent PA phosphatases, which, unlike the LPPs (14, 18), do not have significant activity against other lipid phosphates. These results are compatible with work on yeast Pah1p, which is unable to dephosphorylate DAG pyrophosphate (16).

Our results show that lipin-1, -2, and -3 exhibit distinct tissue expression patterns. The analysis of PAP1 activity in tissues from fld mice establishes lipin-1 as the predominant PAP1 in adipose tissue and skeletal muscle. These results are consistent with a related study by Harris et al. (24) that was published online while this manuscript was in revision. They demonstrated that heart, lung, kidney, and skeletal muscle from fld...
mice lack PAP1 activity (24), but they did not examine PAP1 activity in adipose tissue. Our results demonstrate that lipin-1 is the primary PAP1 enzyme in both white and brown adipose tissue, thus explaining the impaired TAG accumulation and resulting lipodystrophy in the fld mutant mouse. The PAP1 activity of lipin-1 also explains the observed increase in adipocyte size and TAG accumulation in transgenic mice with enhanced lipin-1 expression in adipose tissue (8, 9). Lipin-1 expression was also detected at high levels in human adipose tissue, in agreement with what others and we have observed (25–27). We have recently demonstrated that human adipose tissue lipin-1 mRNA levels vary substantially among individuals in the human population, and that relationships exist between lipin-1 expression and body mass index and insulin sensitivity (25, 26). We, therefore, exercise caution about whether the very high levels of lipin-2 in the anonymous adipose tissue sample we examined is representative of the general population.

The role of lipin-1 as the muscle PAP1 is likely related to the altered fat metabolism and whole body energy expenditure observed in fld mice and muscle-specific lipin-1 transgenic mice (8). The inability to store TAG in fld skeletal muscle may lead to an adaptive response to increase fatty acid disposal, leading to the observed increase in muscle fatty acid oxidation and reduced respiratory quotient in fld mice. Likewise, increased lipin-1 expression in transgenic muscle results in increased TAG accumulation, reduced fatty acid oxidation, and energy expenditure (8).4

In addition to TAG synthesis, PAP1 activity is required for the synthesis of PC and phosphatidylethanolamine. The fact that myocytes and pre-adipocytes develop in the fld mice despite a total lack of PAP1 activity, therefore, raises the question as to how DAG is formed from PA to allow phospholipid synthesis to occur. Our results establish that adipose tissue and skeletal muscle have normal levels of LPP activity, which also catalyzes DAG formation from PA. However, LPPs and PAP1 exhibit nonoverlapping subcellular localization. The catalytic sites of the LPPs are localized to the external surface of the plasma membrane or the luminal surface of internal membranes (14). By contrast, PAP1 activity is localized in the cytosol, and it transiently interacts with the cytosolic surface of the endoplasmic reticulum, the major site of glycerolipid synthesis (28). For the LPPs to substitute for PAP1 in phospholipid synthesis, PA synthesized de novo would have to access the active sites of the LPPs, and the DAG formed would need to translocate to the site of glycerolipid synthesis.

We determined that the lipin-1A and -1B isoforms both exhibit PAP1 activity in a cell-free assay system. The observation that lipin-1B has higher apparent specific activity than lipin-1A suggests that the 33 amino acids that are unique to the B isoform may influence structural or catalytic properties. Although it is unknown whether lipin-1A residing in the nucleus of a living cell also acts as a PAP1 enzyme, there is precedence for the localization of another phospholipid biosynthetic enzyme in the nucleus. This is the case for CTP-phosphocholine cytidylyltransferase-α, which regulates the production of CDPcholine required for the conversion of DAG to PC (29). Alternatively, recent data suggest that lipin in the nucleus of yeast acts as a transcriptional regulator, probably by controlling PA concentrations (23). In mammals, lipin-1 binds to and enhances the activity of the PPARα nuclear receptor and the

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4 K. Reue, unpublished observation.
Mammalian Lipins Are Phosphatidate Phosphatases

PGC-1α coactivator in transcription of a subset of PPARα target genes in liver (30). It remains to be determined whether a similar role for lipin-1 exists in other tissues, such as adipose tissue, and the target genes affected. So far, lipin-1 has been shown to induce gene expression during adipocyte differentiation (5, 9).

The PAP1 activity and expression profiles of lipin-2 and lipin-3 suggest that these lipin family members represent critical PAP1 activities in tissues such as liver, placenta, and brain. Interestingly, all three lipins are expressed to some degree in the digestive tract, including esophagus, stomach, and small intestine. This lipin activity may have a role in the synthesis of membrane phospholipids required for the rapid turnover of the intestinal epithelium. Furthermore, the glycerol phosphate pathway and PAP1 activity are critical for the re-esterification and absorption of fatty acids by enterocytes of the small intestine of ruminants, where TAG is digested completely to glycerol (31). In non-ruminants, the glycerol phosphate pathway is responsible for the re-esterification of about 20% of dietary TAGs because the re-esterification of monoacylglycerol is the major contributor to TAG synthesis. The higher expression of lipin-2 in these regulatory steps on PAP1 activity.

It is notable that lipin-1-deficient mice appear not to have an obvious defect in intestinal morphology or fat absorption. However, the physiological significance of lipin expression in intestine remains to be determined. It is likely that lipin-1-deficient fld mice appear compatible with a role in fat absorption. This lipin activity may have a role in the synthesis of membrane phospholipids required for the rapid turnover of the intestinal epithelium. Furthermore, the glycerol phosphate pathway and PAP1 activity are critical for the re-esterification and absorption of fatty acids by enterocytes of the small intestine of ruminants, where TAG is digested completely to glycerol (31). In non-ruminants, the glycerol phosphate pathway is responsible for the re-esterification of about 20% of dietary TAGs because the re-esterification of monoacylglycerol is the major contributor to TAG synthesis. The higher expression of lipin-1-deficient mice appear not to have an obvious defect in intestinal morphology or fat absorption, perhaps because of the presence of the other two lipin family members in this tissue.

The identification of lipin family members as mammalian PAP1 enzymes will lead to better understanding of the tissue-specific regulation of glycerolipid metabolism. It has been known for some time that PAP1 activity is decreased in adipose tissue in starvation and diabetes (32), whereas the activity in liver increases in these conditions (12), but the mechanism for this tissue-specific regulation is not known. PAP1 is resident primarily in the cytosol but is induced to translocate to the endoplasmic reticulum as fatty acid concentrations and PA synthesis increase (33). Lipin-1 is phosphorylated in response to insulin and amino acids (24, 34), suggesting that different levels of phosphorylation modulate PAP1 localization and activity as suggested previously (28, 29). Studies on the yeast lipin homolog have recently identified seven phosphorylation sites that influence both PAP1 activity and ability of lipin to derepress key enzymes involved in phospholipid biosynthesis (23). Studies in progress indicate that the activity of mammalian lipins are regulated at several levels, including gene transcription, mRNA splicing, protein phosphorylation, and protein degradation.6 It will be important to determine the effect of each of these regulatory steps on PAP1 activity.

In conclusion, our results complement and extend those of Han et al. (16) who demonstrated that the yeast lipin ortholog exhibits PAP1 activity. The absence of lipin-1 in white and brown adipose tissue and skeletal muscle of the fld mouse results in a total absence of detectable PAP1 activity in these tissues. The liver PAP1 activity is maintained in fld mice, probably through expression of lipin-2 and lipin-3, which we also demonstrated to exhibit PAP1 activity. This retention of hepatic PAP1 activity is compatible with the fatty liver and hypertriglyceridemia observed in the fld mouse during the neonatal period (30). Lipin-1 serves not only to catalyze an essential reaction in glycerolipid synthesis, but it also acts as a transcriptional regulator. Our work now lays the foundation for studying the tissue-specific expression of different lipins and understanding how this family of enzymes controls glycerolipid synthesis, cell signaling, and cell differentiation.

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