IN EUKARYOTES, triacylglycerol (TAG) is synthesized through two major pathways, the glycerol phosphate pathway and the monoacylglycerol pathway (24). The glycerol phosphate pathway, first described more than half a century ago, is the major pathway utilized by most cell types (12, 64, 65). Acylation of glycerol 3-phosphate occurs through a stepwise addition of fatty acyl groups, each of which is catalyzed by a distinct enzyme (see Fig. 1). By contrast, the monoacylglycerol pathway functions predominantly in small intestine to generate TAG from monoacylglycerol derived from dietary fat (62). Despite the fact that these pathways have been studied for dozens of years, recent molecular, genetic, and physiological studies have contributed new insight into their complexity. Here we focus on the key enzymes in TAG synthesis through the glycerol phosphate pathway (Fig. 1), whereas the monoacylglycerol pathway is reviewed in a separate article in this series (61).

The first committed step in TAG synthesis via the glycerol phosphate pathway is the acylation of glycerol 3-phosphate by glycerol-3-phosphate acyltransferase (GPAT) enzymes, which reside in the endoplasmic reticulum (ER) and mitochondria (reviewed in Refs. 40 and 43). An additional fatty acid is subsequently transferred to lysophosphatidic acid (LPA) by the family of 1-acylglycerol-3-phosphate acyltransferase (AGPAT; also known as LPA acyltransferase) enzymes to produce phosphatidate (reviewed in Refs. 3 and 106). Phosphatidate occupies a branch point in lipid synthesis. It can serve as a precursor of acidic phospholipids or of diacylglycerol (DAG). The conversion of phosphatidate occurs through the action of phosphatidate phosphatase-1 (PAP) enzymatic activity, which is conferred by the lipin family of proteins (reviewed in Refs. 99, 100, and 102). Finally, the resulting DAG is converted to TAG through the action of diacylglycerol acyltransferase (DGAT) enzymes or used in phospholipid synthesis via the Kennedy pathway (reviewed in Refs. 114 and 129). The resulting TAG is used in several metabolic processes, including synthesis of very-low-density lipoproteins in the liver and chylomicrons in the intestine, energy storage as lipid droplets in adipocytes, and milk production in mammary epithelial cells. TAG can also serve as a sink for potentially toxic fatty acids and acyl-CoAs within cells.

This review will focus on the roles of three protein families in triglyceride biosynthesis: the GPAT, AGPAT, and lipin (PAP) enzymes. A recurrent theme is that multiple proteins with similar enzyme activities exist in each of these families. This, in turn, raises questions about the physiological significance of each of the several enzymes within a family. Here we provide background about the similarities between each member of the three protein families and highlight differences in biological function that have been revealed by studies of tissue expression and regulation and the effects of overexpression or deficiency in mouse models and human disease. Given that a vast literature now exists on several of the enzymes in these families, the current review necessarily neglects some aspects, including effects on phospholipid biosynthesis, and readers are referred to the reviews cited above for additional information. The DGAT enzymes are reviewed in a separate article in this series (105).

**GPAT Enzymes**

As shown in Fig. 1, GPAT enzymes (EC 2.3.1.15) catalyze the first step in TAG synthesis in most tissues, the conversion of glycerol 3-phosphate to 1-acylglycerol-3-phosphate. This reaction is catalyzed by two families of enzymes: GPAT1 and GPAT2. GPAT1 is expressed ubiquitously in mammalian tissues, whereas GPAT2 is primarily expressed in liver and adipose tissue. Both enzymes are regulated by the production of fatty acids, particularly those derived from dietary sources.

**AGPAT Enzymes**

AGPAT enzymes (EC 2.3.1.84) catalyze the transfer of fatty acids from DAG to lysophosphatidic acid (LPA) to form phosphatidate. There are three families of AGPAT enzymes: AGPAT1, AGPAT2, and AGPAT3, each with distinct expression patterns and regulation. AGPAT1 is primarily expressed in liver, whereas AGPAT2 and AGPAT3 are expressed in both liver and adipose tissue. AGPAT enzymes are regulated by the production of fatty acids and by insulin, which stimulates their activity.

**Lipin Enzymes**

Lipin enzymes (EC 3.1.3.24) catalyze the conversion of phosphatidate to DAG. There are two families of lipin enzymes: lipin 1 (LIPIN1) and lipin 2 (LIPIN2). LIPIN1 is primarily expressed in liver and adipose tissue, whereas LIPIN2 is expressed in a variety of tissues. Both enzymes are regulated by the production of fatty acids and by insulin, which stimulates their activity.

**References**

of glycerol 3-phosphate and acyl-CoA to 1-acylglycerol-3-phosphate (lysophosphatidate). Because GPAT exhibits the lowest specific activity of enzymes in the pathway, it has been considered to be rate limiting (24), but genetic defects in AGPAT and lipin enzymes suggest that impaired activity at later steps may also be rate limiting (discussed in later sections). Four mammalian GPAT isoforms have been identified by recent molecular identification studies. GPAT1 localizes to the mitochondrial outer membrane, and its activity is resistant to N-ethylmaleimide (NEM), consistent with previously characterized mitochondrial GPAT activity (43). From the characterization of a GPAT1-knockout mouse model, another mitochondrial isoform with NEM sensitivity, GPAT2, was identified and characterized (53, 73, 120). GPAT3 and GPAT4 are ER membrane isoforms initially designated as AGPAT8 (11, 17, 113) and AGPAT6 (11, 23, 75, 85, 118), respectively. Catalytic activity of both GPAT3 and GPAT4 are NEM sensitive, which is consistent with previously characterized microsomal GPAT activity (24). Below, we summarize properties of each of the four GPAT isoforms. Since GPAT1 has been studied in the greatest depth, more detail is provided on this isoform.

**GPAT1**

**GPAT1 protein characteristics.** GPAT1 is a 94-kDa mitochondrial outer membrane protein. It has two transmembrane domains such that both NH2 and COOH termini are oriented to N-ethylmaleimide (NEM), consistent with previously characterized mitochondrial GPAT activity (43). From the characterization of a GPAT1-knockout mouse model, another mitochondrial isoform with NEM sensitivity, GPAT2, was identified and characterized (53, 73, 120). GPAT3 and GPAT4 are ER membrane isoforms initially designated as AGPAT8 (11, 17, 113) and AGPAT6 (11, 23, 75, 85, 118), respectively. Catalytic activity of both GPAT3 and GPAT4 are NEM sensitive, which is consistent with previously characterized microsomal GPAT activity (24). Below, we summarize properties of each of the four GPAT isoforms. Since GPAT1 has been studied in the greatest depth, more detail is provided on this isoform.

**GPAT1**

**GPAT1 protein characteristics.** GPAT1 is a 94-kDa mitochondrial outer membrane protein. It has two transmembrane domains such that both NH2 and COOH termini are oriented toward the cytosol, with a loop in the intermembrane space (24, 43) (Fig. 2). The NH2-terminal domain contains four acyltransferase motifs (Pfam 01553) that are conserved among glycerolipid acyltransferase family members, including GPATs, AGPATs, and a dihydroxyacetone-phosphate acyltransferase. It has been suggested that motifs I and IV are important for catalysis and that motifs II and III are important for substrate binding (see Table 1). The invariant histidine and aspartate in motif I, the
phenylalanine, glycine, and arginine in motif III, and the proline in motif IV are important for catalysis. The phenylalanine and arginine in motif II and the glutamate and serine in motif III are important for binding to glycerol 3-phosphate (24, 43). The COOH-terminal domain is also necessary for enzyme activity and appears to physically interact with the NH2-terminal domain to contribute to catalysis or substrate binding (94).

Rat GPAT1 is the only mammalian GPAT that has been purified to homogeneity (117), which is due in part to protein instability. In biochemical studies, saturated fatty acyl-CoAs are preferred approximately twofold over unsaturated fatty acyl-CoAs as GPAT1 substrates (117). Esterification occurs at the sn-1 position of glycerol 3-phosphate. Physiological evidence of a preference for C16:0 fatty acyl-CoAs comes from studies of mice with deficiency or knockdown of hepatic GPAT1, in which liver phosphatidylcholine (PC) and phosphatidylethanolamine (PE) contain reduced amounts of C16:0 and increased C18:0 and C18:1 (46, 123). Interestingly, PC and PE in Gpat1−/− liver also contain 40% more C20:4 at the sn-2 position, suggesting that esterification at the sn-2 position is influenced by fatty acids at the sn-1 position (46). GPAT1 overexpression in liver of mice or rats led to increased incorporation of C16:0 fatty acids into LPA, DAG, and TAG (76, 84), consistent with biochemical studies.

The crystal structure of GPAT from squash chloroplast has been determined and has provided information about the structure-function of the GPAT enzymes. The protein is organized into two compact domains: a small domain consisting of the 77 amino-terminal residues and a larger domain (residues 85–368 of the squash enzyme) linked by a seven-aminoc acid loop region (115). A key feature of the large domain is a cleft, appearing as a tunnel lined predominantly by hydrophobic residues. Through an analysis of the crystal structure and amino acid sequence conservation among species, Turnbull et al. (115) proposed glycerol 3-phosphate and fatty acyl substrate binding sites in the large domain. A putative binding site for the phosphate moiety of glycerol 3-phosphate was identified at one end of the cleft, where five positively charged residues (His139, Lys193, His694, Arg335, and Arg337) cluster together in the three-dimensional structure. Turnbull et al. (115) proposed that the fatty acyl substrate lies within a highly evolutionarily conserved hydrophobic patch that lines the surface of the cleft. Both the glycerol 3-phosphate and fatty acyl substrate binding sites are immediately adjacent to the H(X4)D motif (see motif I in Table 1), consistent with the previous identification of this motif as critical for GPAT catalysis (56).

Regulation of GPAT1 gene expression and activity. GPAT1 expression levels are highest in adipose tissue and liver, followed by muscle, brain, kidney, and lung (24, 120). In most tissues, only 10% of total GPAT activity can be attributed to mitochondrial GPAT. In liver, however, mitochondrial GPAT constitutes 30–50% of total activity and has a large impact on the regulation of TAG synthesis (24). GPAT1 is regulated at both the transcriptional and posttranscriptional levels. The activation of GPAT1 is closely associated with the events that require de novo TAG synthesis, suggesting a role for this enzyme in initiating TAG synthesis. Thus, Gpat1 mRNA levels increase >20-fold in mouse liver in an insulin-dependent manner by refeeding of a high-carbohydrate diet after fasting, which is associated with active hepatic lipogenesis. In contrast, fasting for 48 h decreases GPAT1 protein and activity in rat liver and adipose tissues (24). The induction of Gpat1 mRNA by insulin is mediated through the expression of sterol regulatory element-binding protein-1 (SREBP-1), and glucagon counteracts the insulin action through the elevation of cAMP (43). The mouse Gpat1 gene promoter region contains three sterol regulatory elements responsible for SREBP-1-mediated transactivation. Ectopic expression of SREBP-1c in 3T3-L1 adipocytes or in liver of transgenic mice dramatically increases Gpat1 mRNA (24). Treatment with agonist for the nuclear receptor LXR also increases Gpat1 mRNA levels and, this occurs independently of the carbohydrate response element-
binding protein, suggesting that carbohydrate feeding induces hepatic Gpat1 expression through insulin-mediated induction of SREBP-1c (21). Gpat1 mRNA levels also increase during adipocyte differentiation through the transcription factors NF-Y and SREBP-1 (24).

**GPAT1 enzyme activity is affected by insulin and AMP-activated protein kinase.** Insulin treatment of rat primary adipocytes acutely increases the $K_m$ and $V_{\text{max}}$ of GPAT1 for its substrates, which may be mediated through protein phosphorylation (16). In contrast, AMP-activated protein kinase (AMPK), a sensor of cellular energy availability, inhibits GPAT1 activity in concert with the inhibition of acetyl-CoA carboxylase, 3-hydroxy-3-methylglutaryl-CoA reductase, and glycogen synthase, which are key enzymes in synthesis of fatty acids, cholesterol, and glycogen, respectively (24, 43). AMPK also activates carnitine palmitoyltransferase I and enhances $\beta$-oxidation by lowering the concentration of malonyl-CoA. This coordinated regulation suggests that GPAT1 is a critical regulatory point for TAG synthesis in hepatic energy homeostasis. It is not known, however, whether GPAT1 itself or an inhibitor is phosphorylated by AMPK.

**GPAT1 modulates fatty acid oxidation.** GPAT1 is a key determinant of the fate of the cellular fatty acyl-CoA pool in the mitochondrial outer membrane in liver. It is thought that GPAT1 and carnitine palmitoyltransferase I compete for the same long-chain acyl-CoA substrates and channel them toward either glycerolipid biosynthesis or $\beta$-oxidation. Both GPAT gain- and loss-of-function studies support this concept. Thus, GPAT1 overexpression in rat primary hepatocytes results in the increased incorporation of exogenous fatty acids into TAG and phospholipids and reduced rate of $\beta$-oxidation (74, 77). On the other hand, GPAT1-deficient mice fed a high-fat/high-sucrose diet have reduced hepatic TAG but increased plasma $\beta$-hydroxybutyrate and liver acylcarnitine levels, suggesting enhanced $\beta$-oxidation (47, 87). In the high-fat-fed GPAT1-deficient mice, elevated $\beta$-oxidation is associated with increased hepatic acyl-CoA content and activation state of AMPK. These results suggest that enhanced $\beta$-oxidation represents increased energy flow to fatty acid oxidation caused by a blockage of the glycerolipid synthesis pathway. In GPAT1 overexpressing mice, liver fatty acid oxidation measured ex vivo is decreased (76). In GPAT1-overexpressing rats, however, hepatic acyl-CoA content and plasma $\beta$-hydroxybutyrate concentration are similar to those of control rats (84).

As a consequence of enhanced fatty acid oxidation, cells accumulate reactive oxygen species, which may damage cellular components such as proteins, membrane lipids, and DNA. Due to the enhanced $\beta$-oxidation in Gpat1$^{+/−}$ mice, liver mitochondria from these animals exhibit a 20% increase in the rate of reactive oxygen species production and a markedly increased sensitivity to the induction of the mitochondrial permeability transition, an indicator of mitochondrial dysfunction (45). The increased oxidative stress is associated with increased hepatocyte apoptosis and compensatory proliferation, as evidenced by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling-positive staining and bro-modeoxyuridine incorporation. In addition, Gpat1$^{+/−}$ mouse liver contains fewer glutathione-S-transferase-positive cells and lack of induction of expression of DNA repair genes, suggesting lower protection level against oxidative stress, which could increase the risk for liver carcinogenesis (45).

Role of GPAT1 in hepatic TAG production and systemic insulin resistance in vivo. Female Gpat1$^{+/−}$ mice have reduced body weight, gonadal fat pad mass, hepatic TAG content, plasma TAG, and secretion of VLDL-TAG (46). GPAT1 deficiency also protects mice against diet-induced hyperlipidemia and hepatic steatosis (87). After high-fat diet feeding for 3 wk, Gpat1$^{+/−}$ mice exhibit 30% lower plasma TAG levels than wild-type mice, and liver TAG and DAG are reduced, with a reciprocal increase in hepatic acyl-CoA content and plasma $\beta$-hydroxybutyrate concentration, which is consistent with the role of GPAT1 in hepatic glycerolipid synthesis. Feeding Gpat1$^{+/−}$ mice a high-fat/high-sucrose diet produces similar results, except for the failure to cause reduced plasma TAG levels (47). TAG and acyl-CoA content in skeletal muscle is not altered in Gpat1$^{+/−}$ mice. Hepatic shRNA adenovirus-mediated knockdown of GPAT1 in ob/ob mice also results in reduced hepatic TAG and DAG content (123).

The effect of GPAT1 deficiency in insulin resistance has been studied in two diet-induced insulin resistance models. After 1 wk of high-fat feeding, Gpat1$^{+/−}$ mice show improved glucose tolerance and, after 3 wk, are protected from hepatic insulin resistance (87). In the same mice, insulin-stimulated 2-deoxyglucose uptake increases 30% in white adipose tissue but does not change in skeletal muscle. In the other model, Gpat1$^{+/−}$ mice fed a high-fat/high-sucrose diet for 4 mo develop more severe glucose intolerance and hyperinsulinemia than wild-type mice fed the same diet (47). This is associated with impaired suppression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression. The metabolic differences in Gpat1$^{+/−}$ mice fed the two different dietary regimens are not understood but may be related to differences in the composition of dietary fat (safflower oil vs. coconut oil), time on the diet, or other unknown factors.

The overexpression of GPAT1 in mouse and rat liver by adenovirus has been shown to cause hepatic steatosis, hyperlipidemia, and hepatic and systemic insulin resistance (76, 84). An interesting finding is that increased flux through the pathway of de novo hepatic TAG synthesis for relatively short time periods can cause hepatic and systemic insulin resistance in the absence of obesity, a lipogenic diet, or hepatic inflammation. Thus, liver-directed overexpression of GPAT in mouse increases hepatic TAG and DAG content 12- and sevenfold, respectively, and TAG secretion from liver twofold (76). Glucose and insulin levels are not altered. Similar studies in the rat revealed two- to threefold elevations in hepatic TAG and DAG content, a fourfold elevation in LPA, and a fourfold increase in VLDL-TAG (84). Liver glycerogen and plasma glucose levels are reduced slightly, and insulin levels, body weight, and fat pad mass are unchanged. Studies with hyperinsulinemic euglycemic clamp revealed both hepatic and whole body insulin resistance, with 40% lower glucose infusion rate, 22% lower hepatic glucose incorporation into glycerogen, and 2.5-fold higher glucose output from liver (84). In addition, muscle 2-deoxyglucose uptake and glucose incorporation into glycerogen are reduced, and TAG accumulation is increased. The hepatic insulin resistance in GPAT1-overexpressing rats is associated with the activation of PKCε but occurs without evidence of inflammation (84). The effects of GPAT1 overexpression in rat liver are consistent with the hypothesis that elevated DAG levels activate PKCε and contribute to insulin...
resistance (43). However, these animals also exhibit reduced glucose and unaltered insulin levels, raising the possibility that hepatic and peripheral insulin resistance might also be an adaptive response to decreased systemic availability of glucose, more of which is converted to TAG in liver.

**GPAT2**

GPAT2 activity was first identified as the mitochondrial GPAT activity remaining in Gpat1/−/− mice (73). In contrast to GPAT1, GPAT2 activity is sensitive to NEM and heating at 40.5°C, is inhibited by dihydroxyacetone phosphate and polymixin B, is not activated by acetone, and lacks preference for C16:0 fatty acids. GPAT2 activity constitutes 60% of GPAT activity in purified mitochondria from wild-type mouse liver. Molecular identification of GPAT2 was based on amino acid sequence similarity to GPAT1 and calculated molecular mass of the protein (53, 120). Mouse (53) and human GPAT2 (GenBank accession no. NP_997211) have calculated molecular masses of 89 and 88 kDa, respectively, with ~30% amino acid identity to GPAT1.

Compared with GPAT1, the GPAT2 active site region has similar sequences of motifs I and IV and markedly different sequences of motifs II and III (Table 1). From transmembrane domain prediction algorithms based on hydrophobicity and active site domain structure, two transmembrane domains are proposed (Fig. 2). The membrane topography suggested from the position of transmembrane domains is completely different from that of GPAT1. In GPAT2 both the NH2 and COOH termini are located in the intermembrane space, and the loop containing the active site faces the cytosol for access to its substrates (120).

**GPAT2 tissue expression.** The tissue distribution of Gpat2 gene expression is distinct from Gpat1. Mouse Gpat2 is expressed at 50-fold higher levels in testis than in liver. Additional tissues with even lower expression levels than liver include adipose tissue, skeletal muscle, brain, adrenal gland, kidney, lung, and heart (120). In contrast to Gpat1, Gpat2 mRNA does not increase in liver of rats refed a high-sucrose diet after fasting, suggesting less contribution of GPAT2 to diet-induced hepatic TAG synthesis (120). Other aspects of the physiological role of GPAT2 are largely unknown.

**GPAT3**

GPAT3 is an enzyme with a controversial identity, and current evidence suggests that it has both GPAT and AGPAT activities. GPAT3 was first classified as an AGPAT enzyme because of high amino acid sequence similarity to known AGPAT1 and -2 proteins (11, 113). However, AGPAT enzyme activity could not be demonstrated, and instead the protein was found to have GPAT activity with NEM sensitivity (17). Very recently, the same protein has been shown to have AGPAT activity and named AGPAT10/GPAT3 (111) and may in fact catalyze both GPAT and AGPAT reactions.

Mouse and human GPAT3 have a predicted mass of 50 kDa and two putative transmembrane domains, consistent with ER-membrane association (Fig. 2). Evidence for GPAT3 localization to the ER includes immunofluorescence and Western blots of subcellular fractions (17, 113). The acyltransferase domain of GPAT3 resides in the COOH-terminal side of the second transmembrane domain, which differs from the topology of GPAT1 and GPAT2 (17). GPAT3 catalyzes the incorporation of a broad range of long-chain fatty acyl-CoAs, including saturated and unsaturated species, into LPA. Acyltransferase activity of GPAT3 is not detected with mono- or diacylglycerol or lysophospholipid substrates, indicating specificity for glycerol 3-phosphate. GPAT3 overexpression in human embryonic kidney (HEK)-293 cells leads to increased incorporation of exogenous oleic acid into TAG but not into phospholipids (17).

**GPAT3 tissue expression.** Mouse Gpat3 mRNA levels are highest in epididymal white adipose tissue and small intestine, moderate in heart, brown adipose tissue, kidney, and colon, and lower in brain, lung, skeletal muscle, spleen, stomach, and liver (17). Different anatomical fat depots show different expression levels, with higher Gpat3 mRNA levels in visceral fat than in subcutaneous fat (118). Human GPAT3 expression shows a somewhat different distribution than mouse, with highest mRNA levels in kidney, heart, skeletal muscle, thyroid, and testis, moderate levels in adipose tissue, lung, and cerebellum, and lower levels in liver, brain, spleen, and other tissues (17). Gpat3 mRNA increases ~60-fold during differentiation of 3T3-L1 preadipocytes to mature adipocytes, suggesting a critical role in adipocytes. Consistent with this, a 60% knockdown of Gpat3 mRNA in 3T3-L1 cells with small interfering (si)RNA results in a 55% decrease in fatty acid incorporation into LPA (17). Gpat1 mRNA levels also show a large induction during 3T3-L1 adipocyte differentiation, suggesting that this isoform also contributes to GPAT activity in adipocytes.

**Physiological regulation of GPAT3.** To date, few studies have addressed the physiological role of GPAT3. Gpat3 mRNA levels in ob/ob mice are decreased by 70% in white adipose tissue and increased twofold in liver compared with wild-type animals. Treatment of ob/ob mice with rosiglitazone, a potent peroxisome proliferator-activated receptor (PPAR)γ agonist, increases Gpat3, but not Gpat1, mRNA in white adipose tissue, suggesting that Gpat3 is a PPARγ target gene in white adipose tissue and may contribute to increased insulin sensitivity in ob/ob mice treated with rosiglitazone (17). Interestingly, GPAT3 overexpression in HEK-293 cells increases phosphorylation of p70 S6 kinase and 4E-binding protein 1 in an mTOR (mammalian target of rapamycin)-dependent manner, suggesting the possible involvement of lipid intermediates of TAG synthesis, such as LPA and phosphatidic acid (PA), in the mTOR pathway (113).

**GPAT4**

Similarly to GPAT3, GPAT4 was initially classified as AGPAT6 based on high amino acid similarity to AGPAT1 and -2 (11, 75, 118). After careful examination of enzyme activity, it was found instead to be a second ER-localized GPAT and renamed as GPAT4 (23, 85). Mouse and human GPAT4 have a predicted molecular mass of 52 kDa. GPAT4 is predicted to have two or three transmembrane helices and a signal peptide of 38 amino acids. The acyltransferase domain resides in the COOH-terminal side of the second transmembrane domain, which is similar to GPAT3 (see Fig. 2) (11, 75). Overexpression of GPAT4 in cultured cells results in increased NEM-sensitive GPAT activity without AGPAT, DGAT, and other lysophospholipid acyltransferase activity (23, 85). When expressed in cultured cells, GPAT4 can utilize a variety of substrates (120).
substrates, including C12:0-, C16:0-, C18:0-, C18:1-, C18:2-, and C20:4-CoA substrates (23, 85). In contrast to other GPATs, GPAT4 overexpression does not increase incorporation of exogenous fatty acids into TAG in HEK-293 and COS-7 cells, suggesting that LPA and PA produced from the GPAT4 pathway may consist of a separate pool from that utilized for TAG synthesis (23, 85). In HepG2 cells, however, GPAT4 overexpression increases cellular TAG content by 20%, suggesting a possible difference between cell types (85).

**GPAT4 tissue expression.** The Gpat4 tissue expression pattern overlaps with that of other GPATs. Mouse Gpat4 mRNA levels are high in brown adipose tissue and testis, moderate in liver, kidney, brain, intestine, and white adipose tissue, and lower in heart and skeletal muscle (23, 118). Histological analysis of a GPAT4-β-gal fusion protein in mice carrying a gene trap allele for Gpat4 revealed prominent expression in the spermatids of the testis, cerebellum, and hippocampus, in the tubular cells of the kidney, and in the subdermal adipose tissue depot (11, 118).

**Physiological function of GPAT4.** Studies using Gpat4−/− mice have addressed the role of GPAT4 in TAG synthesis in specific tissues as well as the systemic consequences on energy balance. In Gpat4−/− mice, NEM-sensitive GPAT activity is reduced by 65% in liver and brown adipose tissue but is normal in gonadal white adipose tissue, suggesting a critical role for this isoform in liver and brown adipose tissue (85). Gpat4−/− mice show normal Gpat1, -2, and -3 mRNA levels in liver, brown adipose tissue, and white adipose tissue but exhibit a twofold increase in Gpat1 mRNA in liver, without an increase in specific GPAT1 enzyme activity (85). Gpat4 expression is induced in mammary gland epithelium during lactation, and the Gpat4−/− mouse has severely impaired lactation (11). GPAT4 deficiency causes reduced size and number of alveoli, reduced numbers of fat droplets in mammary gland, and reduced TAG and DAG content in milk, suggesting a critical role for GPAT4 in the synthesis of milk TAG.

Gpat4−/− mice fed a chow diet have 25% lower body weight than wild-type animals and are resistant to diet-induced obesity and genetic obesity on the ob/ob background (118). Gonadal white adipose tissue mass and plasma leptin levels are reduced in Gpat4−/− mice, and subdermal adipose tissue, which normally expresses high levels of GPAT4, is nearly absent. In association with the reduced fat content in the subdermal layer, skin TAG, DAG, and free fatty acid levels are markedly reduced in Gpat4−/− mice without cholesterol and fur permeability being changed (118). The reduced body weight of Gpat4−/− mice is associated with increased energy expenditure with no change in food intake, activity, body temperature, respiratory quotient, or adaptive thermogenesis in response to cold. The increase in energy expenditure is possibly an adaptation to the absence of an insulating layer of subdermal fat, which normally plays a role in body temperature regulation. GPAT4 deficiency in the mouse does not alter plasma glucose or insulin levels on chow or high-fat diets or the ob/ob background (118). However, plasma and hepatic TAG levels are reduced 45–50% in Gpat4−/− mice fed a chow diet compared with wild-type mice, consistent with the demonstrated contribution of GPAT4 to liver TAG activity (85, 118).

**AGPAT Enzymes.**

The second acylation step in the glycerol phosphate pathway is the conversion of lysophosphatidate to phosphatidate, adding an acyl group to the sn-2 position of the glycerol backbone (Fig. 1). This step is catalyzed by AGPAT enzymes (EC 2.3.1.51). AGPAT1 and AGPAT2 are well-established AGPATs for which the enzyme activity has been validated (72). On the basis of amino acid sequence similarity, AGPATs 3–10 have been reported (2, 7, 72, 75, 111, 113, 128). Although there have been no comprehensive comparisons of AGPAT activities for all of these enzymes, some evidence suggests that AGPAT2 may have the most robust activity, at least in in vitro assay systems. For example, a direct comparison of mouse AGPATs 2–5 revealed that AGPAT2 had at least twofold higher specific activity than the other three enzymes (80) and 30–70% higher activity than AGPAT9 (7). As described in GPAT Enzymes, AGPAT6 and a protein originally designated AGPAT8 (GenID: 84803) were subsequently shown to have GPAT activity (17, 23, 84). The enzyme currently known as AGPAT8 (GenID: 253558) has been shown to have acyl-CoA: lysocardiolipin acyltransferase (ALCAT) activity (2, 18) as well as AGPAT activity (2). AGPAT7 and AGPAT9 (GenID: 79888) are reported as members of the lysophosphatidylcholine acyltransferase family, with slight AGPAT activity (7, 22, 86, 108, 128). AGPAT10, which is identical in sequence to Gpat3, has been reported to have both AGPAT (111) and GPAT activities (17). Thus, the current understanding of the biochemical and biological roles of most of the AGPATs is far from complete. Below, we focus primarily on AGPAT family members for which some studies addressing potential physiological function have been performed.

**AGPAT1.**

Human AGPAT1 and AGPAT2 were cloned and identified on the basis of amino acid sequence homology to E. coli, yeast, and plant AGPATs (8, 33, 110, 119). Human and mouse AGPAT1 have a calculated molecular mass of 32 kDa (67, 121). AGPAT1 has acyltransferase motifs of the type found in GPAT enzymes (Pfam 01553; Table 1) (24). There are four hydrophobic regions of potential transmembrane domains in AGPAT1, and different topological models have been postulated on the basis of the signal peptide cleavage site or position of catalytic motifs (8, 11, 72). Detailed topology and mutagenesis studies suggest the four-transmembrane domain model with acyltransferase motif I located in the cytosol and motif III located in the ER lumen. Motifs I and IV are involved in the acyl-CoA binding and catalysis, whereas motifs II and III are involved in LPA binding. Location of motifs II and III within a hydrophobic region may be required for the binding to hydrophobic acyl acceptors (125). AGPAT activity and ER localization of AGPAT1 have been confirmed by overexpression using E. coli, mammalian cells, and insect cells (8, 67, 110, 121). The lack of acyltransferase activity for several non-LPA substrates has also been established (8, 59, 121). Baculovirus-mediated AGPAT1 overexpression indicates a preference for fatty acid species C12–16:0, C16:1, C18:2, and C18:3, followed by C18:0, C18:1, and C20:4, and poor activity for C20:0 and C24:0 (8, 59).

Interestingly, AGPAT1 can also catalyze ATP-independent acyl-CoA and LPA synthesis from PA, the reverse of the
normal AGPAT reaction (125). This reversible activity may be involved in the regulation of the levels of LPA and PA available to act as signaling molecules. Overexpression of AGPAT1 and AGPAT2 markedly enhances IL-1β-stimulated IL-6 and TNF-α release, with a significant increase in IL-6 and TNF-α mRNA levels in ECV304 cells, suggesting that AGPAT overexpression may amplify cellular signaling from cytokines (121).

**AGPAT1 Tissue Expression and Effects on Adipocyte and Myocyte Metabolism**

Mouse Agpat1 mRNA is expressed in most tissues, including skeletal muscle, heart, liver, lung, kidney, white and brown adipose tissue, brain, spleen, and thymus (67, 110, 118, 121). Studies in which AGPAT1 was stably overexpressed in 3T3-L1 adipocytes and C2C12 myotubes suggest a possible role of AGPAT1 in energy handling in adipose tissue and muscle (104). Thus, enhanced AGPAT1 expression in 3T3-L1 adipocytes promotes exogenous oleate uptake and incorporation into PA with or without insulin and increases oleate incorporation into TAG under a non-insulin-stimulated state. AGPAT1 overexpression also increases insulin-stimulated glucose uptake by >50% and its conversion to lipid by 85%. As for lipolysis, AGPAT1 overexpression suppresses free fatty acid release in basal and isoproterenol-stimulated conditions without changing glycerol release, suggesting a normal rate of lipolysis with increased reesterification of free fatty acids. In C2C12 myotubes, AGPAT1 overexpression increases insulin-stimulated oleate uptake. In contrast to 3T3-L1 adipocytes, AGPAT1 overexpression does not alter glucose uptake in C2C12 myotubes but influences the metabolic fate of glucose, decreasing glycogen synthesis by 30% and increasing lipid synthesis by 33% (104). It remains to be determined whether altered AGPAT1 expression in vivo will have similar effects on adipocyte and myocyte metabolism.

**AGPAT2**

AGPAT2 is the only member of the family that has been directly associated with a human disease, congenital generalized lipodystrophy (1). An AGPAT2-deficient mouse model has also been developed (26), making AGPAT2 the best-studied AGPAT at the physiological level. Human and mouse AGPAT2 have a calculated molecular mass of 31 kDa (80, 121). An alternatively spliced form of AGPAT2 mRNA, encoding a protein of 246 rather than 278 amino acids, is also found in human (121). Human AGPAT2 shows 35% amino acid identity with AGPAT1 (121). AGPAT2 has a hydrophobicity profile similar to AGPAT1, which suggests that it has transmembrane domain(s) for ER membrane anchoring (11, 33, 72, 80, 126). The subcellular localization of AGPAT2 in ER has been confirmed (32, 39). The enzyme activity of AGPAT2 has been confirmed by overexpression in E. coli, insect cells, and mammalian cells (32, 59, 80, 121). The lack of acyltransferase activity for several other substrates [glycerol 3-phosphate, monoaoylglycerol, DAG, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol (LPG), lysophosphatidinositol (LPI), and lysophosphatidylserine (LPS)] indicates the strict substrate specificity for LPA.

The substrate preferences of AGPAT2 have been studied in detail (59). The preferred acyl donor is 1-oleoyl-LPA. Several acyl donors are incorporated, including C14:0, C16:0, C18:1, and C18:2 fatty acids, with lower incorporation of C18:0 and C20:4 CoAs. PC3 prostate tumor cells overexpressing AGPAT2 incorporate C18:1 into lysophosphatidylmethanol, a membrane-permeable LPA analog utilized by AGPAT2 but not by AGPAT1. The AGPAT2-specific inhibitor CT-32501 inhibits this activity. AGPAT2 overexpression in PC3 cells, however, does not increase incorporation of C20:4 into lysophosphatidylmethanol, and C20:4 incorporation is CT-32501 resistant. These results strongly support the preference of AGPAT2 for C18:1 over C20:4 despite an earlier study suggesting higher preference for C20:4 (33, 59). This fatty acid preference suggests that AGPAT2 is involved mainly in de novo glycerolipid synthesis because the sn-2 position of de novo synthesized glycerolipids contains primarily monoenoic and dienoic acyl groups, in contrast to membrane phospholipids, which are significantly enriched in 20:4 at the sn-2 position.

AGPAT2 deficiency causes congenital generalized lipodystrophy in humans. High levels of mouse Agpat2 mRNA are detected in visceral white and brown adipose tissue, liver, heart, and pancreas, with moderate levels in muscle, lung, small intestine, kidney, and spleen and low levels in brain, placenta, and subcutaneous fat (33, 80, 118, 121). Although other AGPAT enzymes are also expressed in adipose tissue, a critical role for AGPAT2 in human adipocyte TAG synthesis in vivo has been established from studies of patients with mutations in the AGPAT2 gene, which causes one type of Berardinelli-Seip congenital generalized lipodystrophy (CGL) (1, 2). CGL is an autosomal recessive disorder characterized by marked lack of adipose tissue since birth, severe insulin resistance, hypertriglyceridemia, hepatic steatosis, and early onset of diabetes (reviewed in Refs. 3–5 and 57). Most AGPAT2 mutations underlying CGL cause near-complete loss of AGPAT activity in vitro (51). CGL patients with AGPAT2 deficiency lack most of the visceral and subcutaneous fat but maintain mechanical adipose tissue such as subcutaneous fat in the palms, soles, and scalp as well as fat in the periartricular and retroorbital regions (107). This suggests that other AGPAT isoforms may be responsible for activity in these mechanical adipose tissue depots.

Consistent with the near-complete absence of adipose tissue, CGL patients show extremely low serum leptin and adiponectin levels (1, 6, 41, 52). Hypoleptinemia contributes to metabolic complications such as insulin resistance, hypertriglyceridemia, and hepatic steatosis. As a consequence, leptin replacement therapy effectively ameliorates the metabolic complications of CGL (34, 91). The mechanism by which AGPAT2 deficiency results in reduced adipocytokine levels is unclear but may be related to the failure to form mature, lipid-loaded adipocytes.

AGPAT2 in adipocyte differentiation. Since AGPAT2 is the predominantly expressed AGPAT isoform in adipose tissue, the lipodystrophy resulting from AGPAT2 deficiency is likely due in large part to impaired TAG synthesis in white adipose tissue (1). However, AGPAT2 may also have a role in adipocyte differentiation, and defects in this process would also result in lipodystrophy. Thus, siRNA-mediated knockdown of AGPAT2 in OP9 adipocytes causes decreased gene expression for adipogenic proteins such as PPARγ and and CAAT/enhancer-binding protein (C/EBPβ) and delayed induction of mature adipocyte markers such as aP2 and glucose transporter-4 (GLUT4) in addition to reduced TAG accumulation (39).
AGPAT2 knockdown also suppresses the induction of other AGPAT genes and increases levels of several phospholipid species during adipocyte differentiation. These results suggest that AGPAT2 modulates cellular phospholipid levels, which may influence the adipocyte differentiation program (39).

Mechanisms of hepatic steatosis in AGPAT2-deficient mice. In addition to impaired adipose tissue storage, CGL patients develop hepatic steatosis and insulin resistance. The mechanisms contributing to these metabolic abnormalities have been elucidated by studies of Agpat2−/− mice (26). These mice confirm that AGPAT2 has a nonredundant physiological role that cannot be substituted by other AGPAT enzymes, because 80% of AGPAT2 deficient pups die by 3 wk of age, potentially due to hyperglycemia and/or hypothermia. Consistent with CGL patients, AGPAT2-deficient mice lack subcutaneous and intra-abdominal white adipose tissue and interscapular brown adipose tissue, having only 2% body fat compared with intra-abdominal white adipose tissue and interscapular brown adipose tissue and interscapular brown adipose tissue. CGL patients, AGPAT2-deficient mice lack subcutaneous and intraperitoneal brown adipose tissue, having only 2% body fat compared with wild-type mice. The knockout mice have abnormal energy metabolism, including increased food and water intake, increased oxygen consumption, and reduced activity. These may be secondary effects of reduced leptin levels occurring as a consequence of nearly absent adipose tissue. Despite the lack of fat tissue, Agpat2−/− mice have normal body weight, characterized by increased lean mass and organomegaly affecting liver, kidney, spleen, and intestine.

As observed in CGL patients, Agpat2−/− mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. Male mice exhibited more profound hepatic steatosis than female mice, characterized by a sixfold increase in liver TAG content. Expression levels for several other AGPAT genes were increased in the liver of Agpat2−/− male mice, including Agpatl (1.7-fold), Agpat5 (1.6-fold), and Agpat8 (4.6-fold). However, total AGPAT activity was still reduced by 90%, indicating that AGPAT2 is responsible for most of the enzyme activity in liver. The TAG accumulation in liver was associated with a dramatic induction of monoacylglycerol acyltransferase 1 mRNA (48-fold) and protein (7-fold) in liver of male mice, suggesting that TAG synthesis by the monoacylglycerol pathway is used as an alternative to the glycerol 3-phosphate pathway. Additional lipogenic enzyme levels were also elevated, indicating that an increase in de novo TAG biosynthesis contributes to the fatty liver in Agpat2−/− mice. Levels of phospholipids (PE, PC, and phosphatidylserine) were also elevated in AGPAT2-deficient liver, potentially as a consequence of increased monoacylglycerol pathway activity.

Altered AGPAT2 expression in cancer. AGPAT2 is overexpressed in some human cancers, and its specific inhibition has been investigated as a potential targeted cancer therapy. AGPAT2 inhibitors induce growth arrest, apoptosis, or necrosis in various tumor cell lines, including leukemia, non-Hodgkin’s lymphoma, multiple myeloma, ovarian cancer, and endometrial cancer (25, 31, 58, 68, 92, 109). Antitumor activity of AGPAT2 inhibitors has also been demonstrated in mice bearing tumor xenografts (92, 109). Elevated AGPAT2 expression levels are associated with aggressive histology and poor prognosis of ovarian cancer (28, 88, 109). AGPAT2 inhibitors block the Ras/Raf/Erk and phosphatidylinositol 3-kinase/Akt pathways in angiotensin II-stimulated vascular smooth muscle cells, suggesting the involvement of PA produced by AGPAT2 in these pathways (25).

Additional AGPAT Family Members

To date, most of the members of the AGPAT family have been characterized primarily at the biochemical level. AGPAT3, -4, and -5 have been studied compared with AGPAT1 and -2. The AGPAT activity of AGPAT3, -4, and -5 is <50% that of AGPAT2 when oleoyl-CoA is used as the acyl donor, suggesting that other specific acyl-CoAs, or compounds other than LPA, might be more specific substrates for these enzymes (80). These three AGPATs each show distinct tissue expression patterns (79, 80, 118). AGPAT3 mRNA levels are high in brown and white adipose tissue, liver, kidney, and heart, moderate in brain and lung, and low in spleen, epidermis, and skeletal muscle. In contrast, AGPAT4 mRNA levels are extremely high in brain, moderate in lung, intestine, kidney, epidermis, and spleen, and low in liver, heart, and adipose tissues. AGPAT5 mRNA levels are high in brain, adipose tissues, and epidermis, moderate in heart, liver, kidney, and lung, and low in spleen.

Treatment with clofibrate, a potent PPARα agonist, increases cardiac AGPAT3 mRNA in wild-type but not in PPARα-deficient mice, suggesting AGPAT3 regulation by PPARα (80). However, the AGPAT activity in heart appears to be dependent only on the levels of AGPAT2 mRNA, which are markedly reduced in PPARα-deficient mice independent of clofibrate treatment (80). AGPAT3, -4, and -5 show relatively high constitutive expression in epidermis. During rat development, AGPAT2 and AGPAT5 mRNA increase in parallel with AGPAT activity and permeability barrier formation. Disruption of the skin permeability barrier induces AGPAT1, -2, and -3 expression, suggesting a possible role for these AGPATs in phospholipid or TAG synthesis required for epidermis permeability barrier restoration (79).

AGPAT8 is most closely related to AGPAT5. Although the two have overlapping tissue expression patterns, AGPAT8 is expressed at the highest levels in heart, kidney, and skeletal muscle, as well as brain, placenta, and small intestine, whereas AGPAT5 is expressed at higher levels in prostate and testis (2). AGPAT8 has only ∼20% the specific activity of AGPAT2 but has also been reported to act as an ALCAT (ALCAT1) (18). As with other AGPATs, AGPAT9 expression is detected in several tissues, with high levels in lung and spleen and moderate levels in brain, heart, and placenta (7). AGPAT9 activity ranges from 22 to 70% of AGPAT2-specific activity for two different protein isoforms expressed from the same gene. Using a green fluorescent protein-tagged protein, AGPAT9 has been localized to the ER in Chinese hamster ovary cells and appears to colocalize to the same ER sites as AGPAT1 (79, 80). AGPAT9 expression is detected in several tissues, with high levels in lung and spleen and moderate levels in brain, heart, and placenta (7). AGPAT9 activity ranges from 22 to 70% of AGPAT2-specific activity for two different protein isoforms expressed from the same gene. Using a green fluorescent protein-tagged protein, AGPAT9 has been localized to the ER in Chinese hamster ovary cells and appears to colocalize to the same ER sites as AGPAT1 (79, 80). AGPAT9 also localizes to the ER and, as described in GPAT Enzymes, has both AGPAT (111) and GPAT (17) activities.
provide DAG for the synthesis of TAG and the phospholipids PC, PE, and phosphatidylserine. The biochemical properties of the enzymes responsible for this reaction, PAP, have been studied for more than 25 years and classified into two types based on enzymatic properties and subcellular localization (reviewed in Ref. 13). PAP (previously known as PAP1) activity is localized in the cytosol and transiently translocates to the ER membrane to encounter PA substrate and catalyze the phosphatase reaction. PAP enzymes are Mg2+-dependent and NEM sensitive and are responsible for most of the PAP activity that occurs in the glyceral phosphate pathway. In contrast, lipid phosphate phosphatase (previously known as PAP2) activity is Mg2+-independent, NEM-resistant, localized to the plasma membrane, and not involved in TAG synthesis through the glyceral phosphate pathway.

Here we will focus on members of the PAP enzyme family, which were identified as lipin-1, -2, and -3 through a combination of biochemical and genetic approaches. The lipin proteins appear to play important tissue-specific roles in TAG synthesis. In addition, lipin-1 has been shown to act as a transcriptional coactivator in the liver. The dual enzymatic and transcriptional functions of lipin-1 make it unique among enzymes of the glyceral phosphate pathway. The following sections highlight our current understanding of the molecular and physiological functions of lipin proteins in mammals. Recent excellent reviews describe the biochemistry and role of the orthologous yeast enzyme PA hydrolase (19, 20).

Identification of the lipin protein family. The gene encoding lipin-1 was initially identified through the study of a spontaneous mouse mutation known as fatty liver dystrophy (fld). The fld mouse exhibits a complex phenotype that includes fatty liver and hypertriglyceridemia during the neonatal period, followed by the appearance of a peripheral neuropathy in adults (69, 70). In addition, these mice exhibit a severe form of lipodystrophy reminiscent of human CGL and develop insulin resistance (101). When the cause of the fld mutation could not be attributed to any known genes, a genetic approach was employed to identify the mutation through high-resolution genetic mapping and positional cloning. A gene rearrangement in the fld strain was identified in a novel gene that was given the name Lipin1 (96). The mutation prevents the synthesis of the ~100-kDa lipin-1 protein.

Two additional lipin family members, lipin-2 and lipin-3, were identified based on an ~45% similarity in predicted amino acid sequence to lipin-1 (96). Furthermore, lipin gene orthologs are conserved across a broad range of eukaryotes, including vertebrates, fruit fly, nematodes, and yeast, which each have a single lipin-related gene, and plants, which have two lipin orthologs. Lipin orthologs in all species have regions with particularly high conservation in regions near the amino- and carboxyl-terminal ends known as the amino-terminal lipin domain (N-LIP) and carboxyl-terminal lipin domain (C-LIP) domains (see Fig. 2). The strong evolutionary conservation of lipin proteins from yeast to mammals suggests that lipin proteins fulfill a fundamental cellular function.

Lipin proteins are PAP enzymes. Although PAP enzyme activity has been studied for more than two decades, the identity of the proteins responsible was unknown until recently. Attempts to purify the mammalian PAP enzymes were unsuccessful because of protein instability and other technical problems. Fortunately, recent studies in the yeast, S. cerevisiae, were more successful. Purification of PAP activity and peptide sequencing of a 91-kDa protein revealed that PAP activity is conferred by the product of the SMP2 gene (now renamed as PAH1), the yeast ortholog of mammalian lipin genes (49). The PAP catalytic motif (DxDxT) is present in the C-LIP domain of lipin proteins in all species, making them members of a superfamily of Mg2+-dependent halid acid dehalogenase enzymes (Fig. 2) (49). Human lipin-1 and mouse lipin-1, lipin-2, and lipin-3 have all subsequently been shown to have PAP activity (29, 49). This activity is specific for PA and has no activity for related lipid phosphate substrates such as LPA, ceramide-1-phosphate, or sphingosine phosphate (29). The DxDxT motif is critical for PAP enzyme function because conversion of the first or second aspartate residue in DxDxT motif to glutamate completely abolishes the PAP activity (37, 49). A naturally occurring glycine to arginine mutation present in the N-LIP domain in the fld2 mouse causes lipodystrophy similar to the fld mouse and also dramatically reduces PAP enzyme activity (48, 54). This suggests that the N-LIP domain also contributes importantly to enzyme conformation or activity.

Lipin-1 exhibits transcriptional coactivator activity. In addition to PAP enzyme activity, lipin-1 has been shown to interact with a transcription factor complex and to enhance transcriptional coactivation. The role of lipin-1 in transcriptional coactivation was uncovered in a mouse model of PPARγ coactivator-1α deficiency, in which fasting failed to induce the hepatic expression of lipin-1, PPARγ, and PPARα target genes involved in fatty acid oxidation (37). It was further demonstrated that lipin-1 physically interacts with PPARα as well as the transcription factors PPARγ, PPARα, hepatocyte nuclear factor 4α, and the glucocorticoid receptor (37). Notably, the interaction of lipin-1 with these transcription factors requires an LxxIL motif in the C-LIP domain (Fig. 2), and the coactivator function can be decoupled from PAP function, because mutations in the DxDxT motif abolish PAP activity but maintain coactivator function. The physiological role of lipin-1 coactivator function in tissues other than liver remains to be determined.

Role of lipin-1 in adipocyte development. Even prior to the determination that the lipin proteins are PAP enzymes, several lines of evidence have indicated an important role for lipin-1 in TAG storage in adipose tissue. Lipin-1 mRNA is expressed at high levels in white and brown adipose tissue and is induced upon differentiation of preadipocyte cell lines into mature adipocytes (96). Lipin-1 deficiency in the fld mouse results in failure to store TAG in adipose tissue even when animals consume the same amounts of chow or high-fat diets compared with wild-type mice (97, 101). In contrast, transgenic expression of lipin-1 specifically in adipose tissue causes enhanced weight gain and fat mass when mice are fed a high-fat/high-carbohydrate diet, and adipocytes store 60% more TAG per cell than in wild-type mice (98). An explanation for the impaired TAG storage in lipin-1-deficient adipocytes and enhanced storage in transgenic adipocytes was provided by the demonstration that white and brown adipose tissue from fld mice exhibit no PAP activity (29, 54). In contrast, liver from fld mice exhibits normal or only partially reduced PAP activity, indicating that lipin-2 and/or lipin-3 may compensate for this activity in liver (29, 54).
In addition to impaired TAG storage, lipin-1-deficient preadipocytes exhibit defects in the expression of key adipogenic genes. Upon induction of adipocyte differentiation, primary mouse embryonic fibroblasts from fld mice fail to express adipogenic transcription factors, such as PPARγ and C/EBPα, that are normally induced very early in the differentiation process prior to the induction of TAG storage (97). An important role for lipin-1 in the expression of PPARγ target genes in adipocytes has been demonstrated. Thus, lipin-1 physically interacts with PPARγ and is recruited to PPARγ response elements upstream of the phosphoenolpyruvate carboxykinase gene (66). It appears that lipin-1 is required for the maintenance of the mature adipocyte phenotype both through its role in TAG synthesis and as an amplifier of transcriptional activity that maintains expression of mature adipocyte genes.

Lipin-1 mRNA levels in adipose tissue correlate with insulin sensitivity in mouse and man. In the mouse, lipin-1 deficiency is associated with insulin resistance, whereas transgenic overexpression of lipin-1 in adipose tissue promotes insulin sensitivity, although the mice have increased adiposity (98, 101). The relationship between lipin-1 levels in adipose tissue and insulin sensitivity has also been demonstrated in several studies in human subjects. In a group of Finnish subjects, lipin-1 mRNA levels positively correlated with low glucose and insulin levels (112). In a study of normal and obese American subjects with either normal or impaired glucose tolerance, a positive correlation was once again observed between lipin-1 expression levels in adipose tissue and glucose tolerance (127). In young healthy American subjects, insulin sensitivity measured by hyperinsulinemic euglycemic clamp showed the same positive correlation of lipin-1 levels with insulin sensitivity (30). Furthermore, lipin-1 levels in these individuals were associated with enhanced fatty acid oxidation gene expression in adipose tissue and with more effective substrate switching from fat to carbohydrate in response to insulin. Finally, a study in obese human subjects revealed that lipin-1B expression levels in liver were reduced in insulin-resistant individuals and subsequently increased in response to weight loss (27).

Several potential mechanisms may underlie the correlation between lipin-1 levels and insulin sensitivity. Increased lipin-1 expression in adipocytes has been shown to promote expression of GLUT4 and to increase glucose uptake (116). It is also possible that enhanced lipin-1 expression in adipose tissue allows more efficient fatty acid trapping through incorporation into stored TAG, thereby preventing lipid deposition in muscle, liver, and other tissues that could compromise insulin action. In healthy human subjects and in mice, lipin-1 levels were positively correlated with fatty acid oxidation gene expression (30, 98), suggesting that lipin-1 may also influence fat utilization in adipose tissue, as it appears to do in liver (37). It is unclear at present how lipin-1 may coordinate the two seemingly disparate processes of TAG synthesis and fatty acid oxidation, but it could potentially relate to regulation of lipin-1 protein localization to ER (PAP activity) or nucleus (transcriptional coactivator function).

The genetic basis for the observed correlation between lipin-1 levels and insulin sensitivity is less clear. In Finnish subjects, LPIN1 polymorphisms were associated with insulin levels and body mass index (112), and in the Quebec Family Study, LPIN1 polymorphisms were also associated with insulin levels as well as resting metabolic rate and obesity-related phenotypes in an age-related manner (78). Additional studies have identified LPIN1 haplotypes associated with risk for metabolic syndrome, systolic blood pressure, and hemoglobin A1C levels (90, 122). Genetic variation in LPIN1 has also been associated with response to thiazolidinedione drugs used to treat insulin resistance (63). However, a meta-analysis of more than 8,000 individuals in the United Kingdom revealed only a nominal association between LPIN1 polymorphisms and body mass index, and no evidence was seen for common variations in LPIN1 underlying metabolic traits (35). It is likely that both genetic variation in the LPIN1 gene and factors that indirectly regulate lipin-1 expression levels contribute to individual variations in lipin-1 levels.

Role of lipin-1 in skeletal muscle and energy metabolism. In addition to adipose tissue, lipin-1 is expressed at very high levels and accounts for all PAP activity in skeletal muscle (29, 96). In whole animal studies, it has also been demonstrated that the levels of lipin-1 in muscle have important effects on energy expenditure (98). Lipin-1-deficient fld mice have increased energy expenditure and enhanced fatty acid utilization, which may contribute to the lipodystrophic phenotype observed in the wake of normal food intake. In contrast, mice with enhanced lipin-1 expression specifically in skeletal muscle have reduced energy expenditure and fatty acid utilization and become obese even when consuming a standard mouse chow diet. The increased whole body energy expenditure observed in fld mice can be reversed by muscle-specific expression of lipin-1, suggesting that lipin-1 expression levels in skeletal muscle can modulate whole body energy balance (98).

Additional evidence of a critical role for lipin-1 in systemic energy balance comes from the identification in fld mice of an abnormality in fuel switching in the fasting–feeding transitions that occur during the diurnal cycle (124). Wild-type animals typically utilize carbohydrates as energy substrates throughout the fed period and switch to fatty acids liberated from adipose tissue upon fasting for more than a few hours. In contrast, the fld mouse relies on fatty acid substrates during the fed state and utilizes carbohydrates during the fasted state. This abnormality in fuel switching is associated with significant alterations in metabolic flux. Thus, fld mice store and consume twice as much glycogen as wild-type mice in both liver and muscle and have reduced hepatic gluconeogenesis. In the fed state, hepatic de novo fatty acid synthesis is elevated 27-fold in fld mice, and the resulting fatty acids are utilized as energy substrates in peripheral tissues. These observations suggest an adaptation to the impaired lipid storage in adipose tissue of fld mice in which reliance on fatty acid synthesis and utilization during the fed state spares glucose for glycogen storage, providing glucose for use during the fasted state. This unusual adaptation in fuel selection and energy conservation in the fld mouse may account for the lack of fatty liver in adulthood despite the fact that fatty liver is typically seen in other mouse models and humans with lipodystrophy.

Additional observations also implicate lipin-1 in skeletal muscle metabolism. Very recently, mutations in human lipin-1 have been shown to cause acute recurrent myoglobinuria in childhood, reflecting a critical role for lipin-1 in human muscle (described in more detail below) (130). In addition, lipin-1 expression levels in muscle are increased in several distinct conditions that cause muscle atrophy, including diabetes, cachexia, uremia, and prolonged fasting in the mouse (71). It is...
not known whether increased lipin-1 levels are a cause or a consequence of muscle atrophy in these circumstances. Taken together, the studies described above suggest a role for lipin-1 in directing nutrients toward energy storage or utilization and in maintenance of normal muscle function and energy balance.

Regulation of lipin-1 expression and activity. Numerous studies outlined above indicate that lipin-1 expression levels in adipose tissue, skeletal muscle, and liver have important metabolic consequences. Therefore, it is of interest to identify mechanisms that regulate lipin-1 levels and action. Lipin-1 function is regulated at several levels, including mRNA transcription, mRNA splicing, protein phosphorylation, and subcellular localization. In vivo, hepatic lipin-1 gene expression is induced by prolonged fasting and also exhibits circadian regulation, with a peak in hepatic lipin-1 mRNA levels at the end of the light (fasted) period (37, 93). This regulation is abolished by adrenalectomy, suggesting the involvement of glucocorticoids (89). Indeed, recent studies have demonstrated that the induction of lipin-1 expression during adipocyte differentiation in vitro and may also influence mRNA splicing to the lipin-1A and -1B forms (95, 131).

In hepatocytes, the transcriptional induction of lipin-1 by glucocorticoids is further enhanced by cAMP and is attenuated by insulin (81). These results are consistent with early observations that hepatic PAP activity is altered in conditions with elevated glucocorticoids, including diabetes, hypoxia, and fatty liver (14). Although all three mammalian lipin genes are expressed at varying levels in liver, the effect of glucocorticoids is specific for the Lipin1 gene because Lipin2 and Lipin3 are unresponsive (81). Hepatic lipin-1 mRNA levels are also responsive to dietary fatty acid composition. In a study in which mice were fed a low-fat diet (4.8% fat), in which fatty acid type was varied, saturated fatty acids dramatically increased lipin-1 mRNA levels several-hundred-fold, whereas lipin-1 was negatively regulated by polyunsaturated fats (82). This effect requires PPARα, since it does not occur in PPARα-deficient mice. These results implicate lipin-1 levels as one of the factors that contribute to increased hepatic TAG levels in response to dietary fat.

Lipin-1 PAP activity is modulated by factors that influence its subcellular localization. Prior to the identification of the lipin proteins, it was demonstrated that PAP is localized primarily to the cytosol and in the presence of fatty acids or acyl-CoAs translocates to the ER membrane, where it encounters its PA substrate (42). After the identification of lipin-1, it was shown that the protein is phosphorylated in response to insulin and amino acids through the mTOR pathway (60) and that insulin promotes lipin-1 cytosolic localization in 3T3-L1 adipocytes (54). A potential mechanism by which insulin might influence lipin-1 translocation is through phosphorylation at specific serine and threonine residues. Multiple phosphorylation sites on the lipin-1 protein have been identified, including three serine residues (Ser106, Ser634, and Ser209) that are conserved in all three mammalian lipin family members; the Ser106 site was also shown to be responsive to insulin (54). However, much remains to be learned about how protein phosphorylation may regulate lipin translocation, because phosphorylation of Ser106 in response to insulin does not appear to enhance lipin-1 translocation or alter the PAP-specific activity.

Lipin-2 and lipin-3. As described earlier, lipin-2 and lipin-3 were identified through sequence similarity to lipin-1 and have similar protein size and domain organization (reviewed in Ref. 102). Consistent with the conservation of the DxDxT motif, both lipin-2 and lipin-3 exhibit PAP enzyme activity, although specific activity is approximately one-fourth that seen for lipin-1A in a 293T-cell overexpression system (29). The conservation of the LxxIL motif in lipin-2 and lipin-3 suggests that these family members may also interact with nuclear receptors. An interaction between lipin-3 and PPARα has been demonstrated in vitro, although to date there is no information about whether this interaction has a physiological function (37).

The three lipin family members exhibit distinct tissue expression patterns. Whereas lipin-1 appears to be the only PAP in adipose tissue and muscle, lipin-2 is expressed at high levels in liver, brain, and kidney, and lipin-3 expression is detected only at low levels in several visceral tissues, with most consistent expression throughout the small intestine and liver (29). Despite the prominence of lipin-1 in adipose tissue, a role for lipin-2 has also recently been demonstrated in the differentiation of cultured adipocytes. Lipin-2 expression in adipocytes is regulated in an manner opposite to lipin-1, with highest levels in preadipocytes, which subsequently diminishes as cells mature (44). Little is known about the regulation of lipin-3 expression aside from the observed fourfold higher levels of lipin-3 mRNA in the liver of lipin-1-deficient fld mice compared with wild-type animals, suggesting that lipin-3 may contribute to the normal levels of hepatic PAP activity observed in these mice (29).

Lipin gene mutations and human disease. Clear evidence for unique roles of lipin-1 and lipin-2 proteins in human physiology comes from the identification of mutations in LPIN1 and LPIN2. Zeharia et al. (130) identified mutations in the lipin-1 coding sequence that lead to autosomal recessive acute myoglobinuria in childhood in patients of Arab, Palestinian, French, and Mauritanian origin. Individuals homozygous for nonsense or deletion mutations in the lipin-1 coding sequence develop normally but experience episodes of myoglobinuria as children. Somewhat surprisingly, lipodystrophy was not reported in these children, suggesting that this phenotype may develop later in life or not at all in humans, in contrast to the lipin-1-deficient mouse model. The lipid content of muscle from one individual with apparent lipin-1 deficiency was found to have normal levels of PC, PE, and cardiolipin but increased PA, the substrate for lipin-1 enzyme activity. These findings are in line with a study in lipin-1-deficient mice in which Nadra et al. (83) demonstrated PA accumulation in Schwann cells and linked this to the observed peripheral neuropathy observed in these mice. These observations reveal that disease phenotypes associated with lipin-1 deficiency may relate not only to lack of the products generated by PAP activity but also to the accumulation of lipid intermediates. A potential effect of PA accumulation due to lipin-1 deficiency is the inappropriate activation of cell-signaling pathways (reviewed in Ref. 15).

Mutations in human LPIN2 have also been characterized. LPIN2 has been identified as the mutant gene in Majeed syndrome, a rare inflammatory disorder characterized by congenital recurrent multifocal osteomyelitis, cutaneous
inflammation, fever, and dyserythropoietic anemia (9, 36). Three different LPIN2 mutations were identified in three independent families with Majeed syndrome. One of the mutations results in a single amino acid substitution (Ser^{224}Leu) at a position within the C-LIP domain, downstream of the PAP active site and nuclear receptor-binding motif (see Fig. 2), further emphasizing the critical role of the C-LIP domain in lipin protein function. The mechanism by which lipin-2 deficiency results in the abnormalities associated with Majeed syndrome is not known, but possibilities include the loss of PAP activity and/or the accumulation of lipid intermediates in tissues where lipin-2 is the primary PAP enzyme. A single nucleotide polymorphism in the 3′-untranslated region of LPIN2 has also been associated with type 2 diabetes and fat distribution in the Dutch population, although the mechanism by which alterations in lipin-2 might influence these traits has not been established (10). At this point, virtually nothing is known about the physiological function of lipin-3. Gene knockout models in the mouse will likely be instrumental in future elucidation of the in vivo roles for lipin-2 and lipin-3.

GPATs, AGPATs, and Lipins in TAG Synthesis: Key Questions and Future Directions

The role of TAG synthesis in metabolism has received great attention in the past few years, related in part to prevalent disease conditions such as obesity, hyperlipidemia, hepatic steatosis, and insulin resistance. There has been substantial progress in the isolation and molecular characterization of the genes encoding TAG biosynthetic enzymes. However, many questions remain. What is the purpose of multiple GPAT, AGPAT, and lipin enzymes with apparently similar activities? One possibility is to ensure adequate activity through built-in redundancy. The occurrence of severe phenotypes resulting from deficiencies in specific enzymes such as AGPAT2, lipin-1, and lipin-2 argues against this possibility. A second possibility is that each member has a specific spatial and temporal tissue expression pattern or distinct subcellular localization. Although this is a possibility, it does not appear to be that simple, because nearly all of these enzymes are known to have overlapping tissue expression patterns with other family members, and there are examples of several with apparently similar subcellular localization. An additional possibility is that each enzyme is specialized for a particular combination of physiological substrates. This is not trivial to determine and will require side-by-side in vitro comparisons of activity for all members of a particular family over a range of acyl donor and acceptor molecules as well as analysis of the consequences of acute deficiency of each member in vivo.

It is clear that the identification of spontaneous mutations in humans and engineered mutations in the mouse will be instrumental in the further elucidation of physiological functions of the GPAT, AGPAT, and lipin proteins. Recent comparisons of humans and mice with deficiencies in AGPAT2 or lipin-1 have provided new insight into the mechanisms by which lack of these enzymes impacts metabolism. These studies have pointed to the requirement of both AGPAT2 and lipin-1 in adipocyte development and function despite the presence of additional family members in adipocytes. They have also revealed important consequences of impaired function that could not be predicted by in vitro studies, such as the induction of alternative pathways for TAG synthesis leading to hepatic steatosis in AGPAT2-deficient mice and the accumulation of PA substrate in tissues of lipin-1-deficient mice and humans, which may contribute to the pathology of lipin deficiency. These studies also point to potential species differences between effects in mice and humans that should be considered in future studies. Nevertheless, the characterization of induced mouse mutations affecting enzymes of the glycerol 3-phosphate pathway will undoubtedly enlighten us further about the roles of these enzymes in TAG and phospholipid synthesis, lipid signaling, and metabolism. Although there have been tremendous strides since Kennedy first identified the glycerol 3-phosphate pathway about 50 years ago, the next 50 years may be just as interesting.

GRANTS

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