thematic review

Thematic Review Series: Glycerolipids

Mammalian glycerol-3-phosphate acyltransferases: new genes for an old activity

Ruth E. Gimeno[1] **and Jingsong Cao**

Cardiovascular and Metabolic Diseases, Wyeth Research, Cambridge, MA 02140

Abstract Glycerol-3-phosphate acyltransferases (GPATs; EC2.3.1.15) catalyze the first step in the de novo synthesis of neutral lipids (triglycerides) and glycerophospholipids. The existence of multiple enzyme isoforms with GPAT activity was predicted many years ago when GPAT activities with distinct kinetic profiles and sensitivity to inhibitors were characterized in two subcellular compartments, mitochondria and microsomes. We now know that mammals have at least four GPAT isoforms with distinct tissue distribution and function. GPAT1 is the major mitochondrial GPAT isoform and is characterized by its resistance to sulfhydryl-modifying reagents, such as N-ethylmaleimide (NEM). GPAT2 is a minor NEM-sensitive mitochondrial isoform. The activity referred to as microsomal GPAT is encoded by two closely related genes, GPAT3 and GPAT4. GPAT isoforms are important regulators of cellular triglyceride and phospholipid content, and may channel fatty acids toward particular metabolic fates. Overexpression and knock-out studies suggest that GPAT isoforms can play important roles in the development of hepatic steatosis, insulin resistance, and obesity; GPAT isoforms are also important for lactation. This review summarizes the current state of knowledge on mammalian GPAT isoforms.—Gimeno, R. E., and J. Cao. **Mammalian glycerol-3-phosphate acyltransferases: new genes for an old activity. *J. Lipid Res.* 2008.** 49: **2079–2088.**

Supplementary key words triglyceride synthesis • phospholipid synthesis • lipogenesis

Glycerol-3-phosphate acyltransferases (GPATs; EC2.3.1.15) use glycerol-3-phosphate and fatty acyl-CoA as substrates to catalyze the formation of lysophosphatidic acid, a precursor for phosphatidic acid, which in turn is required for the biosynthesis of both triglycerides and glycerophospholipids (**Fig. 1**). GPAT activity was first identified in 1953 using guinea pig liver (1). It has since been shown that GPAT activity is present in most organisms examined, including vertebrates, invertebrates, plants, fungi, and some bacteria (2). [It should be noted that many bacterial species utilize the PlsX/Y pathway, which generates lysophosphatidic acid using acyl-phosphate, rather than acyl-CoA or acyl carrier protein, as a substrate (3)]. The first gene encoding an enzyme with GPAT activity was cloned from *Escherichia coli* (*E. coli*) in 1980 (4, 5) and was demonstrated to be an integral membrane protein (PlsB). Mutational analysis of this gene and alignment with several other glycerophospholipid acyltransferases revealed a conserved domain [pfam01553 or COG2937; http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml (6)] that has since been shown to be present in a large number of acyltransferases, including all mammalian GPAT isoforms identified to date (2, 7, 8).

In mammalian cells, enzymatic studies in the 1960s and 1970s distinguished two GPAT isoforms: a microsomal isoform, which is sensitive to sulfhydryl-modifying reagents, such as N-ethylmaleimide (NEM) and iodoacetamide, and a mitochondrial isoform, which is resistant to NEM (2, 9). The mitochondrial and microsomal isoforms could also be distinguished by their substrate preference (mitochondrial, NEM-resistant GPAT was shown to prefer saturated fatty acyl-CoA, while microsomal, NEM-sensitive GPAT did not have a preference), and their different sensitivity to temperature, polymixin B, acetone, salts, and chelators (2, 9). Recent data show that these two enzymatically defined isoforms are in fact encoded by at least four distinct genes, two of which encode mitochondrial isoforms and two of which encode microsomal isoforms (**Table 1**). In most cells, the GPAT-dependent pathway is thought to be responsible for most of de novo synthesis of neutral lipids and glycerophospholipids. It is important to keep in mind, though, that an alternative pathway for de novo formation of lysophosphatidic acid and ultimately triglycerides and glycerophospholipids also exists (Fig. 1) (10). This pathway, localized in peroxisomes, uses dihydroxyacetone phosphate to generate 1-acyl-dihydroxyacetone phosphate, which in turn is reduced by 1-acyl-dihydroxyacetone phosphate reductase to lysophosphatidic acid (10). This pathway

Manuscript received 23 June 2008 and in revised form 23 July 2008.

Published, JLR Papers in Press, July 24, 2008.
DOI 10.1194/jlr.R800013-JLR200

[1] To whom correspondence should be addressed.
e-mail: rgimeno@wyeth.com

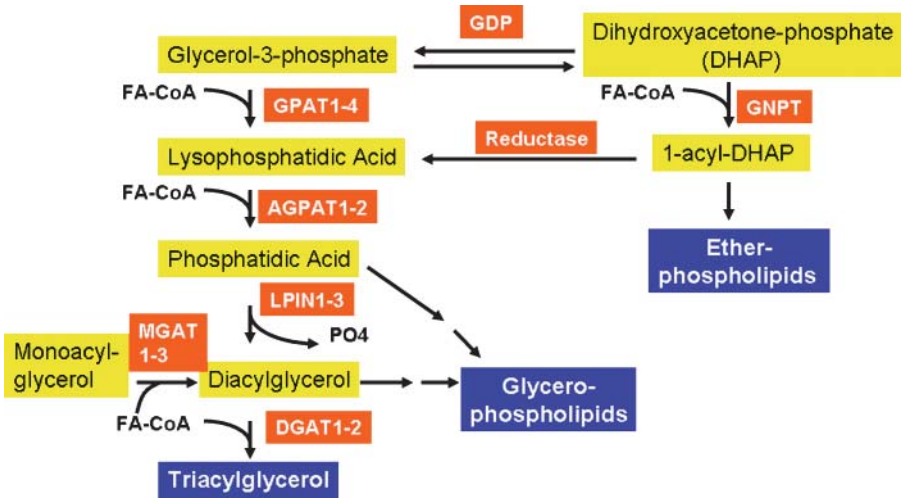

Fig. 1. Glycerolipid biosynthetic pathways. Substrates and intermediates are boxed yellow, major products are boxed blue, and enzymes are boxed red.

is thought to be of only minor quantitative importance for the formation of triglycerides and glycerophospholipids in most tissues, and functions primarily in the formation of ether phospholipids, as demonstrated by studies in tissue culture cells as well as by mutational analysis in mice and humans (11–15). However, it can contribute significantly to glycerolipid formation under certain circumstances (e.g., in differentiated 3T3-L1 adipocytes after an overnight culture in glucose-free medium) (16). It is also noteworthy that diacylglycerol can be produced via the monoacylglycerol pathway in a GPAT-independent manner. The monoacylglycerol pathway utilizes 2-monoacylglycerol and fatty acyl-CoA to generate diacylglycerol (17). In enterocytes, this pathway constitutes a major route for triglyceride formation during fat absorption.

Recent interest in GPATs has been fueled by the recognition that mutations in genes encoding enzymes in the glycerolipid biosynthetic pathway can contribute to or modulate human disease, and that small molecules that inhibit these enzymes may be beneficial for the treatment of disease. For example, genes encoding enzymes in the triglyceride biosynthetic pathway have been shown to be mutated in human and mouse lipodystrophy syndromes (LPIN1, AGPAT2) (18, 19), and polymorphisms in both LPIN1 and LPIN2 genes have been linked to body weight and insulin sensitivity (20). Genes encoding enzymes in the triglyceride biosynthetic pathway have also been linked to inflammatory disorders (LPIN2, AGPAT2) (21, 22) and survival of certain cancers (AGPAT2) (23, 24). Since deletion (25) or inhibition (26) of diacylglycerol acyltransferase 1 (DGAT1) in mouse decreases body weight and improves insulin resistance, there has been intense interest in the development of DGAT1 inhibitors for the treatment of obesity and type 2 diabetes. It might be expected that GPAT isoforms also play a role in human disease and/or could be targets for therapeutics. Consistent with this prediction, knock-outs of two GPAT isoforms in mice, GPAT1 and GPAT4 lead to significant alterations in body weight, insulin sensitivity, and other physiological processes (described in detail below).

TABLE 1. Genes encoding mammalian GPAT isoforms

Gene Name	Alias	Subcellular Localization	Sensitivity to NEM	Accession Number	Reference
GPAT1	mtGPAT1, GPAM	Mitochondria	Resistant	NM_020918 (h) NM_008149 (m) NM_017274 (r)	(31,50)
GPAT2	mtGPAT2, xGPAT1	Mitochondria	Sensitive	NM_207328 (h) NM_001081089 (m) XM_238283 (r)	(68,69)
GPAT3	AGPAT8,[a] AGPAT9,[a] LPAAT-theta	Endoplasmic reticulum	Sensitive	NM_032717 (h) NM_172715 (m) NM_001025670 (r)	(53,81)
GPAT4	AGPAT6, LPAAT-zeta	Endoplasmic reticulum	Sensitive	NM_178819 (h) NM_018743 (m) NM_001047849 (r)	(71–74,85)

LPAAT, lysophosphatidic acid acyltransferases. Mammalian GPAT isoforms identified at the molecular level to date are shown. The gene names listed in column one represents the nomenclature used most recently in the literature. Alternative names are shown in column 2. Accession numbers were assigned based on references cited and on our unpublished sequence analysis.

[a] The names AGPAT8 (86) and AGPAT9 (87) have also been used for the unrelated genes LYCAT (NM_001002257) (88) and LPCAT1 (NM_024830) (89).

TISSUE DISTRIBUTION AND REGULATION OF GPAT ISOFORMS AS DEFINED BY ENZYMATIC STUDIES

Since the information on the recently cloned GPAT isoforms is still limited, it is useful to review the body of literature examining the properties of GPAT isoforms in different tissues, using enzymatic studies. These studies found that the mitochondrial isoform accounts for ~50% of total GPAT activity in rat liver and is significantly decreased upon fasting (27, 28). The microsomal isoform is the major isoform in brown and white adipose tissue, accounting for 80–90% of total GPAT activity (29, 30), and is dramatically upregulated during adipocyte differentiation (16, 31, 32). Microsomal GPAT is also the major isoform in heart (~70% of total activity) (33, 34), and in brain (35). In skeletal muscle, in contrast, it has been reported that >90% of total GPAT activity is the mitochondrial, NEM-resistant isoform (36). Total GPAT activity was reported to be dramatically (~5-fold) upregulated in the islets of obese rats; however, the contribution of different GPAT isoforms to total GPAT activity in islets has not yet been determined (37).

Mitochondrial and microsomal activites are often but not always regulated differentially. Exercise leads to a significant decrease in mitochondrial, but not microsomal GPAT activity in the liver and adipose tissue (36). In rat white adipose tissue, long-term starvation caused a decrease in the activity of mitochondrial, but not microsomal GPAT activity (38). Similarly, starvation or AMP-dependent kinase activation decreases hepatic mitochondrial but not microsomal GPAT activity (28, 39). In contrast, both mitochondrial and microsomal GPAT activities are increased in the liver of diet-induced or genetically obese mice (40). In cold-exposed rats, mitochondrial and, to a lesser extent, microsomal GPAT activity is upregulated in brown adipose tissue (41–43). Increases in microsomal GPAT activity have also been reported in adipose tissue and small intestine of obese rats compared with lean controls (44). While hypothyroidism leads to an increase in microsomal and a decrease in mitochondrial GPAT activity in brown and white adipose tissue, streptozotocin-induced diabetes decreases both activities (30, 42, 43).

MITOCHONDRIAL NEM-RESISTANT GPAT (GPAT1)

Mitochondrial NEM-resistant GPAT (GPAT1) was the first mammalian GPAT isoform to be cloned. In an attempt to identify genes induced in livers of fasted mice refed a high-carbohydrate diet, Sul et al. (45, 46) identified a 6.8 kb mRNA encoding a protein with molecular mass of 90 kDa, that shared sequence similarity with bacterial GPAT. Subsequent overexpression, localization, and knock-out studies confirmed the identity of this protein as a mitochondrial GPAT (now named GPAT1) (31, 47–49). GPAT1 when overexpressed in mammalian or insect cells results in a 1.5–4-fold and 32-fold increase in mitochondrial NEM-resistant GPAT activity, respectively (47, 50). When purified from insect cells, GPAT1 is characterized by a specific activity (~200 nmol/min/mg protein) and apparent K_m for glycerol (0.67 mM) that is comparable to the enzyme purified from rat liver (specific activity of 50–150 nmol/min/mg, K_m for fatty acyl-CoA 3–15 uM, K_m for glycerol of 0.5–1 mM) (47, 51). Knock-out in mice revealed that GPAT1 is responsible for all of the NEM-resistant GPAT activity in liver (48, 49). In the heart, however, significant, NEM-resistant GPAT activity is detectable in GPAT1 knock-out mice, suggesting the existence of a second NEM-resistant GPAT that remains to be identified (34).

GPAT1 mRNA in the rat is most highly expressed in liver, adipose tissue, and oxidative skeletal muscle (52), but is also detectable in significant amounts in many other tissues, including brain, kidney, heart, and adrenal gland. In the mouse, GPAT1 mRNA is also widely expressed with highest levels in white adipose tissue, lung, liver, and heart (53). Interestingly, human GPAT1 is most abundantly expressed in adipose tissue (>10-fold higher compared with other tissues), raising the possibility that in humans, GPAT1 may make a more important contribution to adipose tissue glycerolipid synthesis compared with mice (53). To date, no antibodies specifically recognizing GPAT1 exist. While a discordance between GPAT1 mRNA and protein expression was noted (52), the antibody used in these studies was later shown to cross-react with GPAT2 (49); thus, at least some of the discordance observed may be explained by more significant expression of GPAT2 protein in tissues such as heart.

GPAT1 is transcriptionally regulated in a manner consistent with an important role in triglyceride biosynthesis. Rat liver GPAT1 mRNA increases in response to refeeding after a fast and is increased upon insulin treatment of diabetic, streptozotocin-treated mice (46). In 3T3-L1 adipocytes, GPAT1 mRNA increases upon differentiation (31, 53) and upon treatment with insulin (45), and decreases upon treatment with cAMP mimetics (45). cAMP mimetics also prevent the refeeding-induced increase in GPAT1 mRNA in rat liver (46). The promoter for mouse (54, 55) and rat (56) GPAT1 has been cloned and characterized. A binding site for SREBP-1 in the promoter of mouse GPAT1 has been identified, that is likely important for the upregulation of GPAT1 during adipocyte differentiation (55).

GPAT1 is also regulated posttranslationally via phosphorylation and possibly other events. Casein-kinase 2 stimulates GPAT1 activity by ~50% (57) and has been shown to directly phosphorylate GPAT1 in vitro (58). AMP-dependent kinase has been demonstrated to decrease mitochondrial GPAT activity by ~50% in an ATP-dependent manner as early as 10 min after addition to rat liver mitochondria (39), while insulin increases both the K_m and the V_{max} of GPAT1 in rat adipocytes (59).

GPAT1 plays an important role in hepatic lipid metabolism. In GPAT1 knock-out mice, liver triglycerides and VLDL secretion as well as plasma triglycerides and cholesterol are decreased (48, 60–62), while rats (63) or mice (64) overexpressing GPAT1 in the liver have increased hepatic and serum triglycerides and increased triglyceride secretion. Adenoviral knock-down of GPAT1 in genetically obese ob/ob mice similarly decreased liver triglycerides

but had no effect on plasma triglycerides (65). GPAT1 has been suggested to direct fatty acyl-CoA away from fatty acid oxidation and toward triglyceride biosynthesis, possibly by competing with CPT-1 for fatty acyl-CoA substrate on the outer mitochondrial membrane. In vitro experiments show that overexpression of GPAT1 in hepatocytes increases incorporation of fatty acids into triglycerides and decreases fatty acid oxidation (40, 66). Similarly, in vivo, GPAT1 overexpression leads to decreased hepatic fatty acid oxidation and increased triglyceride biosynthesis (64). In GPAT1 knock-out mice, hepatic fatty acid oxidation rates and serum ketone bodies are increased (60, 61), consistent with a preferential utilization of fatty acids through β-oxidation. Interestingly, however, the substrate of GPAT1, fatty-acyl-CoAs, are also increased in the livers of GPAT1 knock-out mice (60, 62), suggesting that not all fatty-acyl-CoA can be utilized efficiently in the β-oxidation pathway in the absence of GPAT1. It is noteworthy that the phenotypes of GPAT1 knock-out mice are more pronounced in females compared with males, suggesting a possible gender difference in GPAT1 action (48, 61).

Since hepatic steatosis has been linked to insulin resistance, it might be expected that GPAT1 knock-out mice have improved insulin sensitivity. On the other hand, fatty acyl-CoAs and their metabolites have been suggested to be mediators of insulin resistance. Analysis of glucose metabolism in GPAT1 knock-out or overexpression animals suggests complex, diet-dependent effects. GPAT1 knock-out mice fed a normal diet did not show any difference in insulin sensitivity (48). Insulin resistance was increased in GPAT1 knock-out mice compared with wild-type controls, when mice were fed either a high-fat, high-sucrose diet (60), or a diet high in coconut oil (61), but decreased, when mice were fed a high-fat diet rich in safflower oil (62). The decrease in insulin resistance on the high-fat diet rich in safflower oil occurred despite an increase in total hepatic fatty acyl-CoA (62). Knock-down of GPAT1 in ob/ob mice, a model of type 2 diabetes, showed no significant improvement in glucose homeostasis (65). Overexpression of GPAT1 in mice (64) or rats (63) showed no effect (64) or an impairment in insulin sensitivity (63), respectively. It is likely that multiple alterations in hepatic lipid metabolism occur upon GPAT1 modulation that are integrated together, leading to either improved or impaired glucose metabolism, depending on dietary conditions. Hepatic diacylglycerol and lysophosphatidic acid content are increased, and protein kinase ε is activated in GPAT1 overexpressing rats, possibly explaining the insulin resistance in these animals (63). These parameters are decreased in GPAT1 knock-out mice fed a high-fat safflower diet, which may account for their insulin sensitivity, despite high fatty acyl-CoA levels (62). GPAT1 knock-out mice have been reported to have increased oxidative stress in the liver, including increased reactive oxygen species (possibly secondary to increased β-oxidation), increased mitochondrial lipid peroxidation, decreased glutathione-S-transferase levels, and increased hepatocellular apoptosis and proliferation (67). This increase in oxidative stress, which is observed even in animals fed a low-fat diet, may also contribute to the development of insulin resistance in GPAT1 knock-out mice.

Although initial studies suggested decreased body weight in female GPAT1 knock-out mice (48), subsequent experiments showed that GPAT1 deletion did not protect mice from weight gain on a high fat diet (61, 62). Similarly, adenoviral knock-down of GPAT1 in genetically obese ob/ob mice did not decrease their body weight (65). Overexpression of GPAT1 had no significant effect on weight gain in either mice (64) or rats (63). Thus, despite a significant increase in fatty acid oxidation in GPAT knock-out mice, inhibition of GPAT1 is unlikely to result in substantial effects on body weight.

A recent study examined the role of GPAT1 in cardiac lipid metabolism (34). Although GPAT1 accounts for less than 30% of cardiac GPAT activity, GPAT1-deficient mice have decreased cardiac triglyceride, decreased palmitate incorporation into heart triglyceride, and altered fatty acid composition of cardiac phospholipids (34). These changes suggest that modulation of GPAT1 activity may affect cardiac function, although this remains to be demonstrated.

MITOCHONDRIAL NEM-SENSITIVE GPAT (GPAT2)

The existence of a second mitochondrial, NEM-sensitive, GPAT was first recognized when highly purified mitochondria from GPAT1 knock-out mice were found to have a large amount of NEM-sensitive GPAT activity that was not detectable in wild-type mice, presumably representing compensatory upregulation of a second mitochondrial GPAT isoform (49). This activity, termed GPAT2, showed a substrate and inhibitor profile similar to microsomal GPAT, but could be distinguished from microsomal GPAT by its ability to cross-react with antibodies raised against mtGPAT1 (49). The gene encoding this activity was cloned based on its relatively high sequence similarity to mtGPAT1 (27% identity; 70% similarity), and was termed GPAT2 (68, 69). When overexpressed in mammalian cells, GPAT2 localizes to mitochondria and confers an ~30% increase in NEM-sensitive GPAT activity (68, 69). GPAT2 mRNA is most abundant in testis (~50-fold higher compared with other tissues), but is also detectable in most tissues, with highest expression in liver and brown adipose tissue. Unlike GPAT1, GPAT2 mRNA in the liver is not regulated by fasting (68). Little is known about the function of GPAT2. It is remarkable, however, that the phenotypes of GPAT1 knock-out mice occur, even though ~60% of wild-type activity is provided by GPAT2 in the mitochondria. In adipose tissue and liver of normal rats, GPAT2 is likely only a minor component of total GPAT activity, since careful fractionation studies in these tissues have shown <10% NEM-sensitive GPAT activity associated with mitochondria (27–30). However, it is important to keep in mind that GPAT2 may contribute more significantly to total GPAT activity in other tissues and that measurement of NEM-sensitive GPAT activity in unfractionated tissues may assess mitochondrial GPAT2 in addition to microsomal GPAT isoforms.

MICROSOMAL NEM-SENSITIVE GPAT3 (GPAT3)

The first microsomal GPAT (GPAT3) was identified by searching for genes that contain the presumptive acyltransferase active site motif (PF01553) and are highly expressed in adipose tissue and upregulated during adipocyte differentiation (53). GPAT3 shares no significant similarity with GPAT1, and is more closely related to lysophosphatidic acid acyltransferases. However, when overexpressed in mammalian or insect cells, GPAT3 causes a significant (2–6-fold) increase in NEM-sensitive GPAT activity, but no increase in lysophosphatidic acid acyltransferase activity or in acyltransferase activities against a variety of other substrates. Upon overexpression, GPAT3 protein and activity were localized in the endoplasmic reticulum (ER), consistent with a microsomal GPAT (53). Knock-down of GPAT3 in 3T3-L1 adipocytes leads to a significant, ~2-fold, decrease in NEM-sensitive GPAT activity, demonstrating that GPAT3 is a major contributor to GPAT activity in 3T3-L1 adipocytes (53).

GPAT3 mRNA is widely expressed and can be detected in most tissues in both mouse and human (53). In the mouse, GPAT3 mRNA is most abundant in white adipose tissue, small intestine, heart, kidney, and brown adipose tissue. In human, GPAT3 is most abundant in kidney, heart, muscle, and thyroid, but is also present in high levels in adipose tissue, testis, and lung. GPAT3 activity is dramatically upregulated in 3T3-L1 adipocyte differentiation, and is increased in adipose tissue of diabetic mice treated with a PPARγ agonist, raising the possibility that GPAT3 expression is regulated by PPARγ (53). Like many other lipogenic genes, GPAT3 mRNA is also increased in the liver and decreased in white adipose tissue of ob/ob mice (53). Since no antibodies exist that specifically recognize GPAT3, it is unclear whether the expression and regulation of GPAT3 mRNA are mirrored at the protein level. It is possible that GPAT3 activity is also regulated postranslationally by phosphorylation. It has been shown by Nimmo and Houston (70) that the microsomal GPAT activity in adipose tissue is rapidly and almost completely inactivated by addition of protein kinase A and reactivated by alkaline phosphatase, suggesting an inhibitory phosphorylation event either on the microsomal GPAT enzyme itself or on a protein regulating GPAT activity. Multiple potential phosphorylation sites are present in the GPAT3 sequence (53), and it will be interesting to see if any of them are used to regulate the activity of GPAT3.

To date, no knock-outs or mutations in GPAT3 have been described. Since GPAT3 is highly expressed in adipose tissue and small intestine and regulated in a manner similar to other lipogenic enzymes (53), it might be predicted that GPAT3 plays an important role in triglyceride storage.

MICROSOMAL NEM-SENSITIVE GPAT4 (GPAT4)

When GPAT3 was cloned, it was found to be highly related (~80% identity within the acyltransferase domain) to the orphan acyltransferase AGPAT6 (53). Recently studies have shown that AGPAT6 encodes a second microsomal GPAT (71, 72), now named GPAT4. GPAT4, when overexpressed in mammalian cells, confers an ~2-fold increase in NEM-sensitive GPAT activity, while knock-down in cells or knock-out in mice leads to 50–80% decrease in NEM-sensitive GPAT activity (71, 72). Mass spectroscopy experiments directly monitoring the formation of lysophosphatidic acid and phosphatidic acid in mammalian cells incubated with ^{13}C-labeled oleic acid provided convincing evidence that GPAT4 can indeed facilitate the incorporation of oleic acid into lysophosphatidic acid and phosphatidic acid (72). Upon overexpression, GPAT4 protein was localized in the ER, as expected for a microsomal GPAT (72, 73).

Like GPAT3, GPAT4 is widely expressed and can be detected at the mRNA level in most tissues examined (72–74). Mouse GPAT4 is most highly expressed in brown adipose tissue, white adipose tissue, and liver, but is also present at high levels in the testis and in different parts of the brain (73, 74). Human GPAT4 mRNA is ubiquitously expressed (72). Tissues from GPAT4 knock-out mice have been used to estimate the contribution of GPAT4 to total and NEM-sensitive GPAT activity in white and brown adipose tissue and liver of mice (71). Interestingly, while GPAT4 accounts for ~50% of total GPAT activity in brown adipose tissue and liver, GPAT4 knock-out mice had wild-type levels of GPAT activity in white adipose tissue (71).

GPAT4 knock-out mice were generated as part of an effort to develop mutant embryonic stem cell lines using a gene-trapping approach (73, 74). Knock-out mice are viable and fertile, but are unable to nurse their pups. This phenotype is due to a defect in lactation: mammary glands of nursing GPAT4 knock-out females are underdeveloped and contain fewer lipid droplets, and milk triglycerides are decreased by >90%. Since GPAT4 is expressed in mammary gland epithelium and upregulated during lactation, the most likely explanation is that GPAT4 is required for the synthesis of milk triglycerides (73). GPAT4 knock-out mice also show a remarkable decrease in body weight, starting at weaning, which is accompanied by a decrease in body fat and the fat-derived hormone leptin (74). When placed on a high-fat/high-carbohydrate diet or when crossed to genetically obese ob/ob mice, GPAT4 knock-out mice gained significantly less weight compared with their littermate controls. Most strikingly, while significant amounts of inguinal and gonadal fat remained in GPAT4 knock-out mice, subcutaneous adipose tissue was almost completely absent. This suggests that GPAT4 knock-out mice may be a model for selective subdermal lipodystrophy. However, unlike other models of lipodystrophy, which are characterized by hypertriglyceridemia and insulin resistance, GPAT4 knock-out mice have normal or improved serum triglyceride levels and little evidence of severe insulin resistance. The decreased body weight gain of GPAT4 knock-out mice appears to be due to increased energy expenditure, which may be an adaptive response to the lack of an insulating layer of subcutaneous adipose tissue (74). It is interesting that an almost complete absence of subcutaneous adipose tissue is observed in GPAT4 knock-out

mice, even though these mice have wild-type levels of GPAT activities in another white adipose tissue depot, gonadal adipose tissue (71). It will be important to determine the expression of GPAT3 and GPAT 4 in different adipose tissue depots and their contribution to total GPAT activity. Despite the high expression of GPAT4 in brown adipose tissue and liver, there is little evidence for dysfunction in these organs. While the triglyceride content of brown adipose tissue is decreased in knock-out mice, the thermogenic response to cold is normal (74). Liver triglycerides are decreased in GPAT4 knock-out mice in a wild-type background, but are increased when these mice are crossed into the ob/ob background, indicating substantial capacity for liver triglyceride synthesis in absence of GPAT4 (74).

SEQUENCE, STRUCTURAL MOTIFS, AND TOPOLOGY OF MAMMALIAN GPAT ISOFORMS

The bacterial GPAT protein encodes a ~83 kDa protein (8). The two mitochondrial GPAT isoforms cloned to date also encode ~90 kDa proteins, while the microsomal GPAT isoforms are considerably smaller (~50 kDa for GPAT3 and GPAT4). Residues defining the acyltransferase active site are clearly recognizable in all mammalian GPAT isoforms (2, 53, 68, 71, 73) and are located as a block approximately between amino acids 200 and 350 of different GPAT isoforms (**Fig. 2**). Mutations in a number of these residues have been shown to significantly decrease or abolish activity for both mammalian GPAT1 (64, 75) and the bacterial GPAT PlsB (7, 76, 77). Although GPAT1 and GPAT2 are localized to mitochondria when overexpressed (31, 68), neither protein contains a recognizable mitochondrial targeting sequence. The function of the C-terminal extension of GPAT1 and GPAT2 is unclear. While it could be related to mitochondrial targeting, the size of this domain (350 amino acids) suggests additional functions. For example, this domain may serve as a docking site for proteins that functionally interact with or regulate GPAT1 and GPAT 2. Rat adipose tissue GPAT1 has been shown to be phosphorylated at two conserved sites within the C-terminal domain, Ser 632 and Ser 639 (59), although the functional consequences of this phosphorylation have not yet been explored.

All GPAT activities examined to date are tightly associated with membranes. Bacterial GPAT contains no overt transmembrane domains but is inserted asymmetrically into the lipid bilayer, most likely using several amphipathic helixes (8). In contrast, all mammalian GPATs have been predicted to have at least two transmembrane domains. To date, topology has only been examined for GPAT1 (78, 79). The most recent model for GPAT1 topology, based on a combination of protease protection, epitope tagging, and substrate-accessibility studies, suggests two transmembrane domains (Fig. 2) with the N and C terminus as well as the active site facing the cytosol (79). Sequence analysis of GPAT2 also predicts two transmembrane domains; however, only one of these is shared by GPAT1

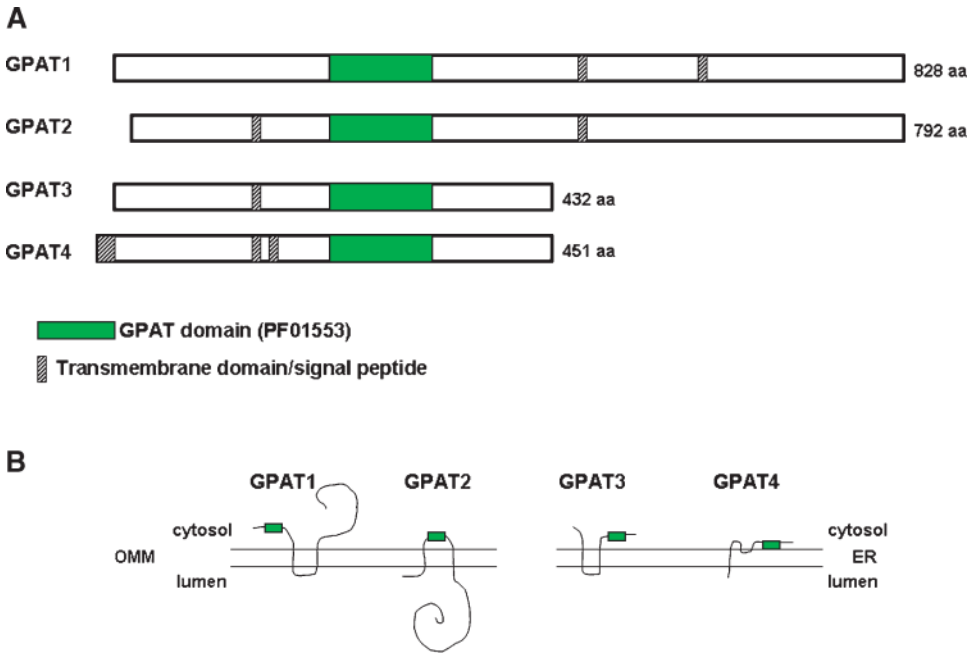

Fig. 2. A: Schematic depiction of mammalian GPAT isoforms. Amino acid numbers represent the human isoforms. The conserved acyltransferase domain is shown in green. Predicted transmembrane domains are shaded. B: Predicted membrane topology of different GPAT isoforms. The topology of GPAT 1 is supported by experimental evidence (79). The topologies of GPAT2, GPAT3, and GPAT4 are unsupported by experimental data and are based entirely on prediction. These predictions take into account the predicted locations of the transmembrane domains as well as data suggesting that the active site of microsomal GPATs faces the cytosol (84). ER, endoplasmic reticulum; OMM, outer mitochondrial membrane.

(68) (Fig. 2). GPAT3 and GPAT4 have been predicted to contain two and three transmembrane domains, respectively (53, 73). The first transmembrane domain is located close to the N terminus and may be cleaved signal sequence in the case of GPAT4. The second (and in the case of GPAT4, third) predicted transmembrane domain is located ~50–70 amino acids upstream of the conserved domain (Fig. 2). The significance of the different locations of the transmembrane domain among different GPAT isoforms is unclear. Given the challenges in predicting membrane domains for integral membrane proteins, it is important to keep in mind, however, that these predictions need to be confirmed experimentally.

ENZYMATIC ACTIVITY OF MAMMALIAN GPAT ISOFORMS

An accurate determination of the inherent enzymatic activity of mammalian GPAT isoforms is made difficult by the instability of these proteins during purification and the requirement for reconstitution in phospholipids, which in itself can affect activity. Only one mammalian GPAT, GPAT1, has been purified to homogeneity to date. Purified rat liver GPAT1 has a specific activity of 50–150 nmol/min/mg, with a K_m for fatty acyl-CoA of 3–15 μM and for glycerol of 0.5–1 mM (51). Similar to GPAT1 in membranes, purified GPAT1 has a preference (~2-fold) for saturated compared with unsaturated fatty acyl-CoA (51). The purified protein has a specific activity that is considerably (~35-fold) lower than what was found for purified GPAT from *E. coli* (51). It is currently unclear whether this difference reflects an inherently lower specific activity of the mammalian enzyme or represents an artifact of the purification process.

The specific activity of GPAT2, GPAT3, and GPAT4 to date has only been assessed in recombinant overexpression systems using either crude lysates or total membrane preparations, making it difficult to compare activities across publications. The reported vector-subtracted increases in GPAT activity upon overexpression are similar to what has been reported for GPAT1, and range from 0.1 to ~1 nmol/min/mg protein for GPAT2 (49, 68, 69), 0.1 nmol/min/mg protein for GPAT3 (53), and 0.4 to 1.5 nmol/min/mg protein for GPAT4 (71, 72).

All GPAT isoforms characterized to date use long-chain fatty acyl-CoA as substrates. While GPAT1 preferentially utilizes saturated fatty acyl-CoA as substrates (2), GPAT2 (49), GPAT3 (53), and GPAT4 (71, 72) appear to be able to utilize saturated and unsaturated fatty acyl-CoA equally well.

WHY SO MANY GPATs?

An interesting question is why mammals have so many different GPAT isoforms. One simple explanation is that multiple genes encoding proteins with redundant functions evolved to allow organisms to compensate in case one gene product is functionally inactivated. However, the phenotypes of GPAT1 and GPAT4 knock-out mice suggest that at least these two isoforms have distinct functions. A second possibility is that multiple genes are required to allow for distinct regulation in different tissues. Since both substrates and products of the GPAT reaction can have potentially deleterious consequences in cells, a tight regulation of GPAT activity coordinately with other factors in the pathway is likely desirable. By providing distinct domains for interacting proteins, and possibly different membrane topologies, GPAT isoforms can be regulated differentially between tissues.

A third explanation takes into account the differential subcellular localization of different GPAT isoforms: it has been suggested that GPAT1 is localized on the mitochondrial outer membrane to be able to channel fatty acyl-CoA away from fatty acid oxidation and into triglyceride biosynthesis. In this scenario, modulation of GPAT1 activity would allow regulated entry of fatty acids into the triglyceride biosynthetic pathway, constituting a check-point analogous to the regulation of fatty acid oxidation by modulation of CPT-1 activity via malonyl-CoA. Consistent with this hypothesis, a negative correlation between GPAT1 activity and fatty acid oxidation is observed in vitro and in vivo (see previous discussion). If this hypothesis is correct, one might also expect that microsomal GPAT isoforms will not show a similar negative correlation between activity and fatty acid oxidation. It will be interesting to see the result of overexpression and knock-out experiments of microsomal GPAT isoforms.

It is also possible that different GPAT isoforms channel substrates into different pathways. Since lysophosphatidic acid is a precursor for triglycerides and phospholipids, it is possible that some GPAT isoforms function primarily in triglyceride biosynthesis, while others are primarily responsible for glycerophospholipid synthesis. Since the final enzymes of triglyceride synthesis are expressed in the ER, it might be expected that microsomal GPATs preferentially generate triglycerides. However, data to date suggest that there is no simple correlation between subcellular localization and the pathway into which the products of the GPAT reaction are channeled. The mitochondrial GPAT isoforms appear to function primarily in triglyceride rather than phospholipid biosynthesis. Overexpression of either GPAT1 (80) or GPAT2 (68) increases triglyceride formation and decreases the formation of most phospholipid species in cells, and GPAT1 overexpression in vivo also increases triglyceride formation (see previous discussion). Overexpression of microsomal GPAT3 shows a phenotype in cells similar to GPAT1 overexpression: incorporation of oleate into triglycerides is increased, while there is little change in the incorporation into phospholipids (53). In contrast, overexpression of microsomal GPAT4 does not increase triglyceride formation but increases the formation of a subset of phospholipids, in particular phosphatidylethanolamine and phosphatidylinositol, while also altering the relative abundance of certain phosphatidylcholine species (71, 72). A simplistic interpretation of these results would suggest that GPAT1, GPAT2, and GPAT3 function in triglyceride synthesis, while GPAT4 functions in phospholipid synthesis.

However, the phenotypes of GPAT4 knock-out mice are not consistent with this notion. The severe decrease in triglyceride content in adipose tissue, liver, skin, and milk, and the mammary gland defect in GPAT4 knock-out mice suggest a role for GPAT4 in triglcyceride biosynthesis, although one cannot exclude the possibility that the observed phenotypes are secondary to a defect in phospholipid synthesis.

Products downstream of GPAT, in particular phosphatidic acid and diacylglycerol, can also function as signaling molecules. Overexpression of GPAT1 (63, 64, 71) or GPAT4 (72) in cells or in vivo have been shown to increase cellular diacylglycerol, lysophosphatidic acid, and phosphatidic acid levels. This increase has been suggested to result in PKCε activation (63). GPAT3 has recently been shown to activate signaling through the mTOR pathway when overexpressed in cells, and it has been suggested that this is mediated by increased levels of phosphatidic acid upon GPAT3 overexpression (81). Thus, certain GPAT isoforms may also regulate signaling pathways under particular circumstances.

While four GPAT isoforms have been identified at the molecular level so far, additional genes encoding proteins with GPAT activity may exist. In particular, recent experiments in GPAT1 knock-out mice suggest the existence of a fifth GPAT isoform in heart which is NEM-resistant and localized on mitochondria (34). It is interesting to note that all genes for GPAT isoforms to date were identified based on the presence of the conserved acyltransferase motif (PF01553). Structurally distinct protein families have been identified that also encode for lipid acyltransferases, in particular the membrane-bound-O-acyltransferase (MBOAT) family, which includes DGAT1 (82) and a recently identified lysophospholipid acyltransferase (83). It will be interesting to see whether additional GPAT encoding genes emerge either from the PF01553 acyltransferase family or the MBOAT family.

PERSPECTIVE

The simple picture of two GPAT isoforms, one mitochondrial and one microsomal, has recently been replaced by the more complex scenario of at least four genes. With the clones of four major GPAT isoforms in hand, we can now begin to ask some important questions: for example, what are the major GPAT isoforms in each tissue, how are they regulated, and which proteins do they interact with? What is the contribution of different GPAT isoforms to the synthesis of glycerolipids in different tissues? Are different GPAT isoforms functionally equivalent, or do they perform different functions? Do GPAT isoforms contribute to human disease, and could the modulation of a particular GPAT isoform be beneficial for treatment? Generation and characterization of knock-out mice for individual GPAT isoforms will be important to begin to develop an understanding of the function of each isoform, and combination of knockouts may be required to fully assess the importance of GPAT isoforms in different tissues. One important area of interest will be the characterization of GPAT isoforms in humans. All enzymatic studies to date have been performed in rodent tissues, and only mRNA expression data is available for human tissues. It will also be interesting to learn more about the nutritional and hormonal regulation of these enzymes and to examine the structure of these proteins in more detail. For example, we currently do not know whether these enzymes form dimers, tetramers, or oligomers in cells; what the critical domains or amino acid residues required for activity are; and how these proteins interact with substrates and channel products in the plane of membrane. The recent cloning of three novel GPAT isoforms has opened the door to start to address these questions.

We would like to thank F. Tremblay and D. Shan for critical reading of this manuscript.

REFERENCES

1. Kornberg, A., and W. E. Pricer, Jr. 1953. Enzymatic esterification of alpha-glycerophosphate by long chain fatty acids. *J. Biol. Chem.* **204:** 345–357.
2. Coleman, R. A., and D. P. Lee. 2004. Enzymes of triacylglycerol synthesis and their regulation. *Prog. Lipid Res.* **43:** 134–176.
3. Zhang, Y. M., and C. O. Rock. 2008. Acyltransferases in bacterial glycerophospholipid synthesis. *J Lipid Res.* **49:** 1867–1874.
4. Lightner, V.A., R. M. Bell, and P. Modrich. 1983. The DNA sequences encoding *plsB* and *dgk* loci of *Escherichia coli*. *J. Biol. Chem.* **258:** 10856–10861.
5. Lightner, V. A., T. J. Larson, P. Tailleur, G. D. Kantor, C. R. Raetz, R. M. Bell, and P. Modrich. 1980. Membrane phospholipid synthesis in Escherichia coli. Cloning of a structural gene (plsB) of the sn-glycerol-3-phosphate acyl/transferase. *J. Biol. Chem.* **255:** 9413–9420.
6. Marchler-Bauer, A., J. B. Anderson, M. K. Derbyshire, C. DeWeese-Scott, N. R. Gonzales, M. Gwadz, L. Hao, S. He, D. I. Hurwitz, J. D. Jackson, et al. 2007. CDD: a conserved domain database for interactive domain family analysis. *Nucleic Acids Res.* **35:** D237–D240.
7. Lewin, T. M., P. Wang, and R. A. Coleman. 1999. Analysis of amino acid motifs diagnostic for the sn-glycerol-3-phosphate acyltransferase reaction. *Biochemistry.* **38:** 5764–5771.
8. Wilkison, W. O., and R. M. Bell. 1997. sn-Glycerol-3-phosphate acyltransferase from Escherichia coli. *Biochim. Biophys. Acta.* **1348:** 3–9.
9. Bell, R. M., and R. A. Coleman. 1980. Enzymes of glycerolipid synthesis in eukaryotes. *Annu. Rev. Biochem.* **49:** 459–487.
10. Hajra, A. K. 1995. Glycerolipid biosynthesis in peroxisomes (microbodies). *Prog. Lipid Res.* **34:** 343–364.
11. Rodemer, C., T. P. Thai, B. Brugger, T. Kaercher, H. Werner, K. A. Nave, F. Wieland, K. Gorgas, and W. W. Just. 2003. Inactivation of ether lipid biosynthesis causes male infertility, defects in eye development and optic nerve hypoplasia in mice. *Hum. Mol. Genet.* **12:** 1881–1895.
12. Gorgas, K., A. Teigler, D. Komljenovic, and W. W. Just. 2006. The ether lipid-deficient mouse: tracking down plasmalogen functions. *Biochim. Biophys. Acta.* **1763:** 1511–1526.
13. Liu, D., N. Nagan, W. W. Just, C. Rodemer, T-P. Thai, and R. A. Zoeller. 2005. Role of dihydroxyacetonephosphate acyltransferase in the biosynthesis of plasmalogens and nonether glycerolipids. *J. Lipid Res.* **46:** 727–735.
14. Ofman, R., E. H. Hettema, E. M. Hogenhout, U. Caruso, A. O. Muijsers, and R. J. Wanders. 1998. Acyl-CoA:dihydroxyacetonephosphate acyltransferase: cloning of the human cDNA and resolution of the molecular basis in rhizomelic chondrodysplasia punctata type 2. *Hum. Mol. Genet.* **7:** 847–853.
15. Declercq, P. E., H. P. Haagsman, P. Van Veldhoven, L. J. Debeer, L. M. Van Golde, and G. P. Mannaerts. 1984. Rat liver dihydroxyacetone-phosphate acyltransferases and their contribution to glycerolipid synthesis. *J. Biol. Chem.* **259:** 9064–9075.
16. Hajra, A. K., L. K. Larkins, A. K. Das, N. Hemati, R. L. Erickson, and O. A. MacDougald. 2000. Induction of the peroxisomal glycerolipid-synthesizing enzymes during differentiation of 3T3–L1 adipocytes. Role in triacylglycerol synthesis. *J. Biol. Chem.* **275:** 9441–9446.

17. Lehner, R., and A. Kuksis. 1996. Biosynthesis of triacylglycerols. *Prog. Lipid Res.* **35:** 169–201.
18. Agarwal, A. K., and A. Garg. 2003. Congenital generalized lipodystrophy: significance of triglyceride biosynthetic pathways. *Trends Endocrinol. Metab.* **14:** 214–221.
19. Reue, K., and P. Zhang. 2008. The lipin protein family: dual roles in lipid biosynthesis and gene expression. *FEBS Lett.* **582:** 90–96.
20. Reue, K., and J. Donkor. 2007. Genetic factors in type 2 diabetes: all in the (lipin) family. *Diabetes.* **56:** 2842–2843.
21. Ferguson, P. J., S. Chen, M. K. Tayeh, L. Ochoa, S. M. Leal, A. Pelet, A. Munnich, S. Lyonnet, H. A. Majeed, and H. El-Shanti. 2005. Homozygous mutations in LPIN2 are responsible for the syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anaemia (Majeed syndrome). *J. Med. Genet.* **42:** 551–557.
22. Leung, D. W. 2001. The structure and functions of human lysophosphatidic acid acyltransferases. *Front. Biosci.* **6:** D944–D953.
23. Diefenbach, C. S., R. A. Soslow, A. Iasonos, I. Linkov, C. Hedvat, L. Bonham, J. Singer, R. R. Barakat, C. Aghajanian, and J. Dupont. 2006. Lysophosphatidic acid acyltransferase-beta (LPAAT-beta) is highly expressed in advanced ovarian cancer and is associated with aggressive histology and poor survival. *Cancer.* **107:** 1511–1519.
24. La Rosee, P., T. Jia, S. Demehri, N. Hartel, P. de Vries, L. Bonham, D. Hollenback, J. W. Singer, J. V. Melo, B. J. Druker, et al. 2006. Antileukemic activity of lysophosphatidic acid acyltransferase-beta inhibitor CT32228 in chronic myelogenous leukemia sensitive and resistant to imatinib. *Clin. Cancer Res.* **12:** 6540–6546.
25. Chen, H. C., and R. V. Farese, Jr. 2005. Inhibition of triglyceride synthesis as a treatment strategy for obesity: lessons from DGAT1-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **25:** 482–486.
26. Zhao, G., A. J. Souers, M. Voorbach, H. D. Falls, B. Droz, S. Brodjian, Y. Y. Lau, R. R. Iyengar, J. Gao, A. S. Judd, et al. 2008. Validation of diacyl glycerolacyltransferase I as a novel target for the treatment of obesity and dyslipidemia using a potent and selective small molecule inhibitor. *J. Med. Chem.* **51:** 380–383.
27. Nimmo, H. G. 1979. Evidence for the existence of isoenzymes of glycerol phosphate acyltransferase. *Biochem. J.* **177:** 283–288.
28. Bates, E. J., and E. D. Saggerson. 1979. A study of the glycerol phosphate acyltransferase and dihydroxyacetone phosphate acyltransferase activities in rat liver mitochondrial and microsomal fractions. Relative distribution in parenchymal and non-parenchymal cells, effects of N-ethylmaleimide, palmitoyl-coenzyme A concentration, starvation, adrenalectomy and anti-insulin serum treatment. *Biochem. J.* **182:** 751–762.
29. Saggerson, E. D., C. A. Carpenter, C. H. Cheng, and S. R. Sooranna. 1980. Subcellular distribution and some properties of N-ethylmaleimide-sensitive and-insensitive forms of glycerol phosphate acyltransferase in rat adipocytes. *Biochem. J.* **190:** 183–189.
30. Baht, H. S., and E. D. Saggerson. 1988. Comparison of triacylglycerol synthesis in rat brown and white adipocytes. Effects of hypothyroidism and streptozotocin-diabetes on enzyme activities and metabolic fluxes. *Biochem. J.* **250:** 325–333.
31. Yet, S. F., S. Lee, H. Young Tae, and S. Hei Sook. 1993. Expression and identification of p90 as the murine mitochondrial glycerol- 3-phosphate acyltransferase. *Biochemistry.* **32:** 9486–9491.
32. Coleman, R. A., B. C. Reed, J. C. Mackall, A. K. Student, M. D. Lane, and R. M. Bell. 1978. Selective changes in microsomal enzymes of triacylglycerol phosphatidylcholine, and phosphatidylethanolamine biosynthesis during differentiation of 3T3–L1 preadipocytes. *J. Biol. Chem.* **253:** 7256–7261.
33. Swanton, E. M., and E. D. Saggerson. 1997. Glycerolipid metabolizing enzymes in rat ventricle and in cardiac myocytes. *Biochim. Biophys. Acta.* **1346:** 93–102.
34. Lewin, T. M., H. de Jong, N. J. Schwerbrock, L. E. Hammond, S. M. Watkins, T. P. Combs, and R. A. Coleman. 2008. Mice deficient in mitochondrial glycerol-3-phosphate acyltransferase-1 have diminished myocardial triacylglycerol accumulation during lipogenic diet and altered phospholipid fatty acid composition. *Biochim Biophys Acta.* **1781:** 352–358.
35. Fitzpatrick, S. M., G. Sorresso, and D. Haldar. 1982. Acyl-CoA: sn-glycerol-3-phosphate O-acyltransferase in rat brain mitochondria and microsomes. *J. Neurochem.* **39:** 286–289.
36. Park, H., V. K. Kaushik, S. Constant, M. Prentki, E. Przybytkowski, N. B. Ruderman, and A. K. Saha. 2002. Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. *J. Biol. Chem.* **277:** 32571–32577.
37. Lee, Y., H. Hirose, Y. T. Zhou, V. Esser, J. D. McGarry, and R. H. Unger. 1997. Increased lipogenic capacity of the islets of obese rats: a role in the pathogenesis of NIDDM. *Diabetes.* **46:** 408–413.
38. Lawson, N., A. D. Pollard, R. J. Jennings, M. I. Gurr, and D. N. Brindley. 1981. The activities of lipoprotein lipase and of enzymes involved in triacylglycerol synthesis in rat adipose tissue. Effects of starvation, dietary modification and of corticotropin injection. *Biochem. J.* **200:** 285–294.
39. Muoio, D. M., K. Seefeld, L. A. Witters, and R. A. Coleman. 1999. AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem. J.* **338:** 783–791.
40. Linden, D., L. William-Olsson, M. Rhedin, A. K. Asztely, J. C. Clapham, and S. Schreyer. 2004. Overexpression of mitochondrial GPAT in rat hepatocytes leads to decreased fatty acid oxidation and increased glycerolipid biosynthesis. *J. Lipid Res.* **45:** 1279–1288.
41. Darnley, A. C., C. A. Carpenter, and E. D. Saggerson. 1988. Changes in activities of some enzymes of glycerolipid synthesis in brown adipose tissue of cold-acclimated rats. *Biochem. J.* **253:** 351–355.
42. Mitchell, J. R. D., and E. D. Saggerson. 1991. Activities of enzymes of glycerolipid synthesis in brown adipose tissue after treatment of rats with the adrenergic agonists BRL 26830A and phenylephrine, after exposure to cold and in streptozotocin-diabetes. *Biochem. J.* **277:** 665–669.
43. Mitchell, J. R. D., and E. D. Saggerson. 1994. The response of brown adipose tissue mitochondrial glycerolphosphate acyltransferase to cold-exposure in hypothyroidism, after adrenalectomy and after treatment with cycloheximide. *Int. J. Biochem.* **26:** 67–72.
44. Jamdar, S. C., and W. F. Cao. 1995. Triacylglycerol biosynthetic enzymes in lean and obese Zucker rats. *Biochimica et Biophysica Acta - Lipids and Lipid Metabolism.* **1255:** 237–243.
45. Paulauskis, J. D., and H. S. Sul. 1988. Cloning and expression of mouse fatty acid synthase and other specific mRNAs. Developmental and hormonal regulation in 3T3–L1 cells. *J. Biol. Chem.* **263:** 7049–7054.
46. Shin, D. H., J. D. Paulauskis, N. Moustaid, and H. S. Sul. 1991. Transcriptional regulation of p90 with sequence homology to Escherichia coli glycerol-3-phosphate acyltransferase. *J. Biol. Chem.* **266:** 23834–23839.
47. Yet, S. F., M. Yangha Kim, and S. Hei Sook. 1995. Purification and reconstitution of murine mitochondrial glycerol-3- phosphate acyltransferase. Functional expression in baculovirus-infected insect cells. *Biochemistry.* **34:** 7303–7310.
48. Hammond, L. E., P. A. Gallagher, S. Wang, S. Hiller, K. D. Kluckman, E. L. Posey-Marcos, N. Maeda, and R. A. Coleman. 2002. Mitochondrial glycerol-3-phosphate acyltransferase-deficient mice have reduced weight and liver triacylglycerol content and altered glycerolipid fatty acid composition. *Mol. Cell. Biol.* **22:** 8204–8214.
49. Lewin, T. M., N. M. J. Schwerbrock, D. P. Lee, and R. A. Coleman. 2004. Identification of a new glycerol-3- phosphate acyltransferase isoenzyme, mtGPAT2, in mitochondria. *J. Biol. Chem.* **279:** 13488–13495.
50. Ganesh Bhat, B., P. Wang, J. H. Kim, T. M. Black, T. M. Lewin, F. T. Fiedorek, Jr., and R. A. Coleman. 1999. Rat sn-glycerol-3-phosphate acyltransferase: molecular cloning and characterization of the cDNA and expressed protein. *Biochim. Biophys. Acta.* **1439:** 415–423.
51. Vancura, A., and D. Haldar. 1994. Purification and characterization of glycerophosphate acyltransferase from rat liver mitochondria. *J. Biol. Chem.* **269:** 27209–27215.
52. Lewin, T. M., D. A. Granger, J. H. Kim, and R. A. Coleman. 2001. Regulation of mitochondrial sn-glycerol-3-phosphate acyltransferase activity: response to feeding status is unique in various rat tissues and is discordant with protein expression. *Arch. Biochem. Biophys.* **396:** 119–127.
53. Cao, J., J. L. Li, D. Li, J. F. Tobin, and R. E. Gimeno. 2006. Molecular identification of microsomal acyl-CoA:glycerol-3-phosphate acyltransferase, a key enzyme in de novo triacylglycerol synthesis. *Proc. Natl. Acad. Sci. USA.* **103:** 19695–19700.
54. Jerkins, A. A., W. R. Liu, S. Lee, and H. S. Sul. 1995. Characterization of the murine mitochondrial glycerol-3-phosphate acyltransferase promoter. *J. Biol. Chem.* **270:** 1416–1421.
55. Ericsson, J., S. M. Jackson, J. B. Kim, B. M. Spiegelman, and P. A. Edwards. 1997. Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1- and sterol regulatory element-binding protein-responsive gene. *J. Biol. Chem.* **272:** 7298–7305.

56. Aneja, K. K., P. Guha, R. Y. Shilpi, S. Chakraborty, L. M. Schramm, and D. Haldar. 2008. The presence of distal and proximal promoters for rat mitochondrial glycerol-3-phosphate acyltransferase. *Arch. Biochem. Biophys.* **470:** 35–43.
57. Onorato, T. M., and D. Haldar. 2002. Casein kinase II stimulates rat liver mitochondrial glycerophosphate acyltransferase activity. *Biochem. Biophys. Res. Commun.* **296:** 1091–1096.
58. Onorato, T. M., S. Chakraborty, and D. Haldar. 2005. Phosphorylation of rat liver mitochondrial glycerol-3-phosphate acyltransferase by casein kinase 2. *J. Biol. Chem.* **280:** 19527–19534.
59. Bronnikov, G. E., N. Aboulaich, A. V. Vener, and P. Stralfors. 2008. Acute effects of insulin on the activity of mitochondrial GPAT1 in primary adipocytes. *Biochem. Biophys. Res. Commun.* **367:** 201–207.
60. Hammond, L. E., S. Neschen, A. J. Romanelli, G. W. Cline, O. R. Ilkayeva, G. I. Shulman, D. M. Muoio, and R. A. Coleman. 2005. Mitochondrial glycerol-3-phosphate acyltransferase-1 is essential in liver for the metabolism of excess acyl-CoAs. *J Biol Chem.* **280:** 25629–25636.
61. Yazdi, M., A. Ahnmark, L. William-Olsson, M. Snaith, N. Turner, F. Osla, M. Wedin, A. K. Asztely, A. Elmgren, Y. M. Bohlooly, et al. 2008. The role of mitochondrial glycerol-3-phosphate acyltransferase-1 in regulating lipid and glucose homeostasis in high-fat diet fed mice. *Biochem. Biophys. Res. Commun.* **369:** 1065–1070.
62. Neschen, S., K. Morino, L. E. Hammond, D. Zhang, Z. X. Liu, A. J. Romanelli, G. W. Cline, R. L. Pongratz, X. M. Zhang, C. S. Choi, et al. 2005. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. *Cell Metab.* **2:** 55–65.
63. Nagle, C. A., J. An, M. Shiota, T. P. Torres, G. W. Cline, Z. X. Liu, S. Wang, R. L. Catlin, G. I. Shulman, C. B. Newgard, et al. 2007. Hepatic overexpression of glycerol-sn-3-phosphate acyltransferase 1 in rats causes insulin resistance. *J. Biol. Chem.* **282:** 14807–14815.
64. Linden, D., L. William-Olsson, A. Ahnmark, K. Ekroos, C. Hallberg, H. P. Sjogren, B. Becker, L. Svensson, J. C. Clapham, J. Oscarsson, et al. 2006. Liver-directed overexpression of mitochondrial glycerol-3-phosphate acyltransferase results in hepatic steatosis, increased triacylglycerol secretion and reduced fatty acid oxidation. *FASEB J.* **20:** 434–443.
65. Xu, H., D. Wilcox, P. Nguyen, M. Voorbach, T. Suhar, S. J. Morgan, W. F. An, L. Ge, J. Green, Z. Wu, et al. 2006. Hepatic knockdown of mitochondrial GPAT1 in ob/ob mice improves metabolic profile. *Biochem. Biophys. Res. Commun.* **349:** 439–448.
66. Lewin, T. M., S. Wang, C. A. Nagle, C. G. Van Horn, and R. A. Coleman. 2005. Mitochondrial glycerol-3-phosphate acyltransferase-1 directs the metabolic fate of exogenous fatty acids in hepatocytes. *Am. J. Physiol. Endocrinol. Metab.* **288:** E835–E844.
67. Hammond, L. E., C. D. Albright, L. He, I. Rusyn, S. M. Watkins, S. D. Doughman, J. J. Lemasters, and R. A. Coleman. 2007. Increased oxidative stress is associated with balanced increases in hepatocyte apoptosis and proliferation in glycerol-3-phosphate acyltransferase-1 deficient mice. *Exp. Mol. Pathol.* **82:** 210–219.
68. Wang, S., D. P. Lee, N. Gong, N. M. Schwerbrock, D. G. Mashek, M. R. Gonzalez-Baro, C. Stapleton, L. O. Li, T. M. Lewin, and R. A. Coleman. 2007. Cloning and functional characterization of a novel mitochondrial N-ethylmaleimide-sensitive glycerol-3-phosphate acyltransferase (GPAT2). *Arch. Biochem. Biophys.* **465:** 347–358.
69. Harada, N., S. Hara, M. Yoshida, T. Zenitani, K. Mawatari, M. Nakano, A. Takahashi, T. Hosaka, K. Yoshimoto, and Y. Nakaya. 2007. Molecular cloning of a murine glycerol-3-phosphate acyltransferase-like protein 1 (xGPAT1). *Mol. Cell. Biochem.* **297:** 41–51.
70. Nimmo, H. G., and B. Houston. 1978. Rat adipose-tissue glycerol phosphate acyltransferase can be inactivated by cyclic AMP-dependent protein kinase. *Biochem. J.* **176:** 607–610.
71. Nagle, C. A., L. Vergnes, H. Dejong, S. Wang, T. M. Lewin, K. Reue, and R. A. Coleman. 2008. Identification of a novel sn-glycerol-3-phosphate acyltransferase isoform, GPAT4, as the enzyme deficient in Agpat6−/− mice. *J. Lipid Res.* **49:** 823–831.
72. Chen, Y. Q., M. S. Kuo, S. Li, H. H. Bui, D. A. Peake, P. E. Sanders, S. J. Thibodeaux, S. Chu, Y. W. Qian, Y. Zhao, et al. 2008. AGPAT6 is a novel microsomal glycerol-3-phosphate acyltransferase. *J. Biol. Chem.* **283:** 10048–10057.
73. Beigneux, A. P., L. Vergnes, X. Qiao, S. Quatela, R. Davis, S. M. Watkins, R. A. Coleman, R. L. Walzem, M. Philips, K. Reue, et al. 2006. Agpat6–a novel lipid biosynthetic gene required for triacylglycerol production in mammary epithelium. *J. Lipid Res.* **47:** 734–744.
74. Vergnes, L., A. P. Beigneux, R. Davis, S. M. Watkins, S. G. Young, and K. Reue. 2006. Agpat6 deficiency causes subdermal lipodystrophy and resistance to obesity. *J. Lipid Res.* **47:** 745–754.
75. Dircks, L. K., J. Ke, and H. S. Sul. 1999. A conserved seven amino acid stretch important for murine mitochondrial glycerol-3-phosphate acyltransferase activity. Significance of arginine 318 in catalysis. *J. Biol. Chem.* **274:** 34728–34734.
76. Heath, R. J., and C. O. Rock. 1998. A conserved histidine is essential for glycerolipid acyltransferase catalysis. *J. Bacteriol.* **180:** 1425–1430.
77. Heath, R. J., and C. O. Rock. 1999. A missense mutation accounts for the defect in the glycerol-3-phosphate acyltransferase expressed in the plsB26 mutant. *J. Bacteriol.* **181:** 1944–1946.
78. Balija, V. S., T. R. Chakraborty, A. V. Nikonov, T. Morimoto, and D. Haldar. 2000. Identification of two transmembrane regions and a cytosolic domain of rat mitochondrial glycerophosphate acyltransferase. *J. Biol. Chem.* **275:** 31668–31673.
79. Gonzalez-Baro, M. R., D. A. Granger, and R. A. Coleman. 2001. Mitochondrial glycerol phosphate acyltransferase contains two transmembrane domains with the active site in the N-terminal domain facing the cytosol. *J. Biol. Chem.* **276:** 43182–43188.
80. Igal, R. A., S. Wang, M. Gonzalez-Baro, and R. A. Coleman. 2001. Mitochondrial glycerol phosphate acyltransferase directs the incorporation of exogenous fatty acids into triacylglycerol. *J. Biol. Chem.* **276:** 42205–42212.
81. Tang, W., J. Yuan, X. Chen, X. Gu, K. Luo, J. Li, B. Wan, Y. Wang, and L. Yu. 2006. Identification of a novel human lysophosphatidic acid acyltransferase, LPAAT-theta, which activates mTOR pathway. *J. Biochem. Mol. Biol.* **39:** 626–635.
82. Cases, S., S. J. Smith, Y. W. Zheng, H. M. Myers, S. R. Lear, E. Sande, S. Novak, C. Collins, C. B. Welch, A. J. Lusis, et al. 1998. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Natl. Acad. Sci. USA.* **95:** 13018–13023.
83. Zhao, Y., Y. Q. Chen, T. M. Bonacci, D. S. Bredt, S. Li, W. R. Bensch, D. E. Moller, M. Kowala, R. J. Konrad, and G. Cao. 2008. Identification and characterization of a major liver lysophosphatidylcholine acyltransferase. *J. Biol. Chem.* **283:** 8258–8265.
84. Bell, R. M., L. M. Ballas, and R. A. Coleman. 1981. Lipid topogenesis. *J. Lipid Res.* **22:** 391–403.
85. Li, D., L. Yu, H. Wu, Y. Shan, J. Guo, Y. Dang, Y. Wei, and S. Zhao. 2003. Cloning and identification of the human LPAAT-zeta gene, a novel member of the lysophosphatidic acid acyltransferase family. *J. Hum. Genet.* **48:** 438–442.
86. Agarwal, A. K., R. I. Barnes, and A. Garg. 2006. Functional characterization of human 1-acylglycerol-3-phosphate acyltransferase isoform 8: cloning, tissue distribution, gene structure, and enzymatic activity. *Arch. Biochem. Biophys.* **449:** 64–76.
87. Agarwal, A. K., S. Sukumaran, R. Bartz, R. I. Barnes, and A. Garg. 2007. Functional characterization of human 1-acylglycerol-3-phosphate-O-acyltransferase isoform 9: cloning, tissue distribution, gene structure, and enzymatic activity. *J. Endocrinol.* **193:** 445–457.
88. Cao, J., Y. Liu, J. Lockwood, P. Burn, and Y. Shi. 2004. A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. *J. Biol. Chem.* **279:** 31727–31734.
89. Nakanishi, H., H. Shindou, D. Hishikawa, T. Harayama, R. Ogasawara, A. Suwabe, R. Taguchi, and T. Shimizu. 2006. Cloning and characterization of mouse lung-type acyl-CoA:lysophosphatidylcholine acyltransferase 1 (LPCAT1). Expression in alveolar type II cells and possible involvement in surfactant production. *J. Biol. Chem.* **281:** 20140–20147.