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Review

Glycerol-3-phosphate acyltransferases: Rate limiting enzymes of triacylglycerol biosynthesis

Angela A. Wendel, Tal M. Lewin, Rosalind A. Coleman *

Department of Nutrition, CB# 7461, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

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ABSTRACT

Four homologous isoforms of glycerol-3-phosphate acyltransferase (GPAT), each the product of a separate gene, catalyze the synthesis of lysophosphatidic acid from glycerol-3-phosphate and long-chain acyl-CoA. This step initiates the synthesis of all the glycerolipids and evidence from gain-of-function and loss-of-function studies in mice and in cell culture strongly suggests that each isoform contributes to the synthesis of triacylglycerol. Much work remains to fully delineate the regulation of each GPAT isoform and its individual role in triacylglycerol synthesis.

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1. Glycerol-3-phosphate acyltransferases are members of the pfam 01553 family of acyltransferases

After Eugene Kennedy and his colleagues showed that the esterification of glycerol-3-phosphate with a long-chain acyl-CoA was the initial step in the synthesis of phospholipids [1] and triacylglycerol (TAG) [2], Pullman's group reported that this *sn*-glycerol-3-phosphate acyltransferase activity (GPAT; EC 2.3.1.15) was comprised of what appeared to be two isoforms, one located in the mitochondrial outer membrane and the other in the endoplasmic reticulum [3]. The endoplasmic reticulum (microsomal) activity was inhibited by sulfhydryl reagents such as *N*-ethylmaleimide (NEM) and exhibited no preference for particular acyl-CoA species, whereas the mitochondrial activity was resistant to NEM inactivation and preferred to use saturated acyl-CoAs like 16:0-CoA and 18:0-CoA [4]. With the identification of four genes encoding separate GPAT isoenzymes [5–11], we now know that GPAT mediated regulation of glycerolipid synthesis is more complex than anyone had previously thought; investigators are currently struggling with the question as to why four separate isoforms are required for glycerolipid biosynthesis.

Gpat1, the first mammalian GPAT isoform cloned [5,6], resides in the outer mitochondrial membrane, is resistant to NEM inactivation and prefers to use saturated acyl-CoAs [4]. A second mitochondrial GPAT, GPAT2, also resides in the outer mitochondrial membrane, but its activity is inhibited by NEM, and it has no long-chain acyl-CoA preference [12]. The NEM-sensitive endoplasmic reticulum isoforms, GPAT3 and GPAT4, were identified very recently [9–11].

All four GPAT isoforms are members of the pfam 01553 family of glycerolipid acyltransferases and contain four conserved motifs first identified by a bioinformatics approach [13] (Fig. 1). Mutagenesis of invariant residues in these motifs verified that these highly conserved regions are essential for the activity of *E. coli* GPAT (PlsB) [14,15], mouse GPAT1 [16], and human dihydroxyacetone phosphate acyltransferase (DHAPAT) [17]. Residues important for catalysis are the invariant histidine and aspartate in Motif I, the phenylalanine, glutamate, glycine, and arginine in Motif III, and the proline in Motif IV [15,16,18]. Amino acids important for binding *sn*-glycerol-3-phosphate are the phenylalanine and arginine in Motif II, and glutamate and serine in Motif III [15,16,18]. A diverse array of acyltransferases have been assigned to pfam 01553 and activities have been verified biochemically for GPAT1–4, *sn*-1-acylglycerol-3-phosphate O-acyltransferase (AGPAT)-1 and -2, DHAPAT, lysophosphatidylcholine acyltransferase (LPCAT)-1, -2 and -3, lysophosphatidylglycerol acyltransferase (LPGAT), and acyl-CoA:lysocardiolipin acyltransferase (ALCAT) [4,17,19–25]. In spite of its homology to the glycerolipid acyltransferases, tafazzin, a protein required for the synthesis of normal cardiolipin, exhibits a transacylase activity [26–28]. A fifth motif present in the AGPAT isoforms, GPAT-3 and -4, and tafazzin has been inferred because amino acid mutations in this region of human AGPAT2 and in tafazzin cause congenital generalized lipodystrophy or Barth syndrome, respectively [29,30]. Amino acids important for the

* Corresponding author. Tel.: +1 919 966 7213; fax: +1 919 843 8555.
E-mail address: rcoleman@unc.edu (R.A. Coleman).

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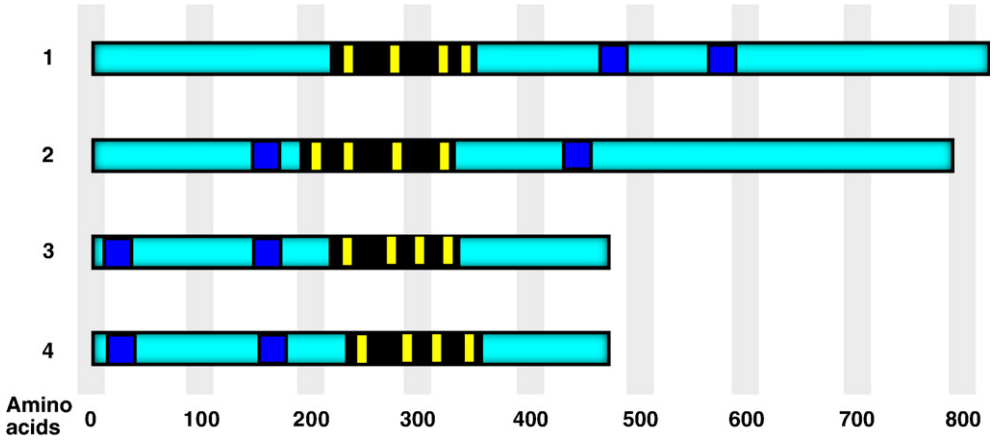

Fig. 1. Domain structure of GPAT1–4. The black regions represent the active site with the four active site motifs in yellow. Putative transmembrane domains (TMDs) are shown as blue squares. Only the TMDs and topography of GPAT1 have been confirmed experimentally; its N- and C-termini face the cytosol and the loop between the TMDs faces the mitochondrial intramembrane space [73].

specialized activities of most of the other pfam 01553 family members have yet to be identified.

2. GPAT1 is an important regulator of TAG synthesis

Although GPAT1 resides in the outer mitochondrial membrane and diacylglycerol acyltransferase, the terminal enzyme of TAG synthesis, resides on the ER, many studies support an important role for GPAT1 in regulating TAG synthesis. GPAT1 activity is highest in rat liver and adipose, tissues with a high capacity for TAG synthesis [12]. In these tissues, GPAT1 activity is modulated in a manner consistent with the regulation of TAG synthesis. For example, in rat liver and adipose tissue, a 48 h fast decreases GPAT1 protein expression and activity more than 30%. When fasted rats are refed sucrose, GPAT1 protein expression and activity increases greater than 2-fold in liver [31,32], and in perfused rat liver, insulin increases GPAT1 activity 34% [33]. Similarly, streptozotocin-induced diabetes decreases GPAT1 activity in rat epididymal fat 60%, and insulin administration restores GPAT activity [34].

Changes in GPAT1 activity are mediated transcriptionally. When fasted mice are refed a high-carbohydrate diet, hepatic *Gpat1* mRNA levels increase 20-fold due to enhanced transcription rates [31]. The refeeding-induced increase in *Gpat1* message occurs in streptozotocin-diabetic mice only if insulin is administered. This regulation of *Gpat1* by insulin is mediated by sterol regulatory element binding protein-1 (SREBP-1), a transcription factor responsible for transactivating numerous genes required for enhanced fatty acid and TAG synthesis [35]. Insulin induces transcription of SREBP-1c, and in a typical counter-regulatory fashion, glucagon via cAMP opposes the action of insulin [36]. SREBP-1c appears to be the primary transcriptional regulator of GPAT1. The mouse *Gpat1* promoter region, first characterized by Jerkins et al. [37], contains three SREBP-1 consensus sites which are responsible for SREBP-1 mediated transactivation [38]. When SREBP-1c is ectopically expressed in 3T3-L1 preadipocytes, *Gpat1* mRNA expression increases 6.7-fold [38]. Conversely, in SREBP-1 knockout mice, the normal 8-fold induction of hepatic *Gpat1* mRNA expression by refeeding is abolished [39]. Although LXR agonists have been reported to upregulate GPAT1, LXR does not directly transactivate *Gpat1*, but rather upregulates SREBP-1c by a transcriptional mechanism [40]. In addition, although *Gpat1* mRNA increases with carbohydrate feeding, there is no evidence that this upregulation is mediated through the carbohydrate response element; instead, it appears to proceed entirely through insulin-mediated transactivation of SREBP-1c since *Gpat1* mRNA expression is similar in wildtype and *Chrebp*$^{-/-}$ mice [41].

The studies summarized above indicate that GPAT1 in liver is regulated in a manner consistent with a role in initiating TAG synthesis when an animal is presented with excess carbohydrate or fat calories. Less information is available about the regulation of GPAT1 in other tissues. *Gpat1* mRNA increases 10-fold when 3T3-L1 cells differentiate into adipocytes [5], coinciding with an increase in TAG synthesis, and mitochondrial GPAT activity (probably GPAT1) decreases 50% in rat epididymal fat and liver, but not in gastrocnemius muscle, after treadmill exercise, probably regulated by AMP-activated kinase (AMPK) [42]. However, NEM-resistant GPAT activity increases in brown adipose tissue (BAT) when animals are exposed to cold [43], suggesting that TAG synthesis may continue simultaneously with TAG lipolysis. Such a futile cycle would contribute to the energy wasting and heat production in BAT. None of these effects, however, has been examined systematically and the controlling factors have not been elucidated.

3. The role of GPAT1 in TAG synthesis

Gain-of-function and loss-of-function studies highlight the importance of GPAT1 in *de novo* TAG synthesis. Plasmid- and adenovirus-mediated overexpression of GPAT1 in CHO and HEK293 cells and in primary rat hepatocytes increases TAG content and oleate incorporation into TAG 3- to 4-fold [44,45]. Further, compared to rats infected with a control virus, when rats are infected with a GPAT1 expressing adenovirus, NEM-resistant GPAT activity, hepatic TAG content, and plasma TAG concentration each increase about 2.7-fold [46]. In contrast, compared to wildtype controls, female mice that lack GPAT1 (*Gpat1*$^{-/-}$) weigh less, have smaller gonadal fat pads, have 40% lower hepatic TAG content, 15% lower plasma TAG, and 30% reduced secretion of VLDL-TAG [47]. Lipogenic diets also result in less TAG accumulation in liver and heart from *Gpat1*$^{-/-}$ mice [48,49,74], although the total weight gain of *Gpat1*$^{-/-}$ and wildtype mice is similar. Consistent with a role for GPAT1 in mediating TAG synthesis, a 90% adenovirus-mediated shRNA knockdown of liver *Gpat1* in *ob*/*ob* mice reduces liver TAG content 30–42% within 5 days [50]. Surprisingly in this model, no change was observed in plasma TAG concentration.

4. GPAT1 regulates acyl-CoA metabolism at the mitochondrial membrane

In addition to controlling the flux of acyl-CoAs that enter the pathway of TAG synthesis, GPAT1 also regulates acyl-CoA use for β-oxidation. Once fatty acids are activated to acyl-CoAs, they can be esterified by GPAT and enter the pathway of glycerolipid biosynthesis or they can be converted to acyl-carnitines by carnitine palmitoyltransferase-1 (CPT1) and then enter the mitochondrion for β-oxidation. GPAT1 and CPT1 are intrinsic proteins of the outer mitochondrial membrane, and appear to compete for the same

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