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Recent progress on acyl CoA: lysophospholipid acyltransferase research

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Abstract Cells of all organisms are enclosed by a plasma membrane containing bipolar lipids, cholesterol, and proteins. Cellular membranes contain several classes of glycerophospholipids, which have numerous structural and functional roles in cells. Polyunsaturated fatty acids including arachidonic acid and eicosapentaenoic acid are usually located at the *sn*-2 position, but not the *sn*-1 position, of glycerophospholipids in an asymmetrical manner. Glycerophospholipids are first formed by the de novo pathway (Kennedy pathway) using acyl-CoAs as donors. Subsequently, in the remodeling pathway (Lands' cycle), cycles of deacylation and reacylation of glycerophospholipids modify the fatty acid composition to generate mature membrane with asymmetry and diversity. Both pathways were proposed in the 1950s. Whereas the enzymes involved in the Kennedy pathway have been well characterized, little is known about the enzymes involved in the Lands' cycle. Recently, several laboratories, including ours, have identified enzymes working in the Lands' cycle from the 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT) family, and also from the membrane bound O-acyltransferases (MBOAT) family. These discoveries have prompted a robust surge of research in this field. In this review, we focus on the cloning and characterization of lysophospholipid acyltransferases (LPLATs), which contribute to membrane asymmetry and diversity.—Shindou, H., D. Hishikawa, T. Harayama, K. Yuki, and T. Shimizu. **Recent progress on acyl CoA: lysophospholipid acyltransferase research. *J. Lipid Res.* 2009.** 50: **S46–S51.**

Supplementary key words Lands' cycle • LPLAT • membrane diversity • membrane asymmetry • glycerophospholipid • platelet-activating factor • MBOAT • AGPAT • remodeling pathway • surfactant lipid

THE BIOSYNTHETIC PATHWAY

Lipids play essential roles in living system (1, 2). Glycerophospholipids are important structural and functional components of biological membranes and constituents of serum lipoproteins and the pulmonary surfactant. Additionally, glycerophospholipids play important roles as precursors of lipid mediators such as platelet-activating factor (PAF) and eicosanoids (2, 3). In each tissue, cellular membranes contain a distinct composition of various glycerophospholipids (1, 4, 5). The acyl groups of glycerophospholipids are highly diverse, depending on the polar head group, and are distributed in an asymmetric manner (4, 6). Saturated and monounsaturated fatty acids are usually esterified at the *sn*-1 position, whereas polyunsaturated acyl groups are esterified at the *sn*-2 position, although several atypical distributions have been reported (1, 2, 4, 6, 7). Using acyl-CoAs, glycerophospholipids are first synthesized from glycerol-3-phosphate in the de novo pathway, originally described by Kennedy and Weiss in 1956 (Kennedy pathway) (8), and undergo maturation in the remodeling pathway, as reported by Lands in 1958 (Lands' cycle) (**Fig. 1**) (9). Rapid turnover of the *sn*-2 acyl moiety of glycerophospholipids is attributed to the concerted and coordinated actions of phospholipase A_2s (PLA_2s) and lysophospholipid acyltransferases (LPLATs) (Fig. 1) (2, 6, 7, 10). Because there are many species of glycerophospholipids differing in the phosphoryl head groups and the fatty acids in chain lengths and degrees of saturation, many LPLATs should exist. Even though these pathways occur in almost all tissues, in the 50 years

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Abbreviations: AGPAT, 1-acylglycerol-3-phophate O-acyltransferase; CL, cardiolipin; GP, glycerol-3-phosphate; GPAT, GP acyltransferase; LCLAT, lyso-CL acyltransferase; LPA, lysophosphatidic acid; LPAAT, lyso-PA acyltransferase; LPCAT, lyso-PC acyltransferase; LPEAT, lyso-PE acyltransferase; LPGAT, lyso-PG acyltransferase; LPIAT, lyso-PI acyltransferase; LPLAT, lysophospholipid acyltransferase; LPSAT, lyso-PS acyltransferase; MBOAT, membrane bound O-acyltransferase; PA, phosphatidic acid; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PLA_2, phospholipase A_2; PS, phosphatidylserine.

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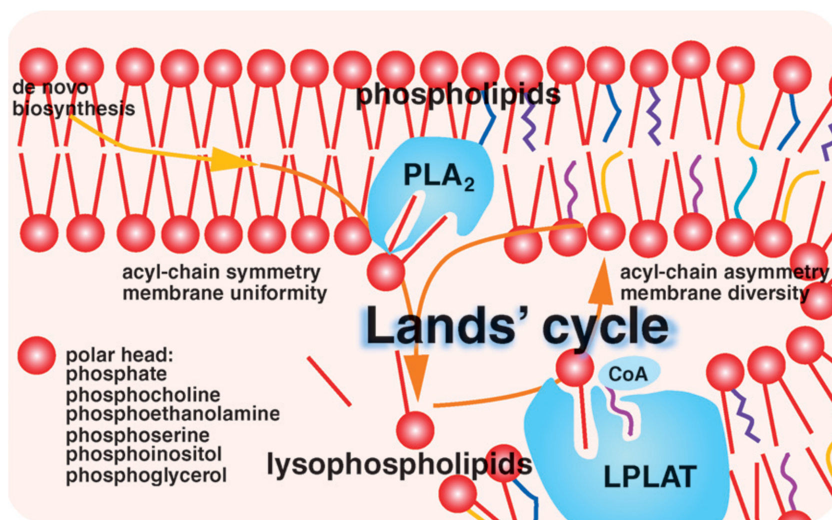

Fig. 1. The biogenesis of membrane diversity. Kennedy and Weiss reported the first synthesis of phospholipids in the de novo pathway (Kennedy pathway), and subsequently Lands reported the maturation of phospholipids in the remodeling pathway (Lands' cycle) to produce membrane diversity. The remodeling pathway is attributed to the concerted and coordinated actions of phospholipase A_2 (PLA_2s) and lysophospholipid acyltransferase (LPLATs).

since Lands' proposal, there has been no information available on acyltransferases involved in phospholipid remodeling. Recently, several LPLATs have been cloned and characterized by several laboratories, including ours (7). In this review, we summarize recent research on the cloning and characterization of the remodeling enzymes.

ACYLTRANSFERASES IN THE DE NOVO PATHWAY (KENNEDY PATHWAY)

In the de novo pathway of glycerophospholipid biosynthesis, lysophosphatidic acid (LPA) is first formed from glycerol-3-phosphate (GP) by glycerol-3-phosphate acyltransferases (GPAT)s (11, 12). LPA is then converted to phosphatidic acid (PA) by lyso-PA acyltransferases (LPAATs), and two types of glycerol derivatives are generated from PA (11, 12). One is diacylglycerol, which is subsequently converted to triacylglycerol, phosphatidylcholine (PC), and phosphatidylethanolamine (PE). Some of them are changed into phosphatidylserine (PS). The other glycerol derivative is cytidine diphosphate diacylglycerol, which is modified to form phosphatidylinositol (PI), phosphatidylglycerol (PG), and cardiolipin (CL), or PS. Many key enzymes in the de novo pathways have been characterized, and more detailed information is available in several recent reviews (1, 11, 12).

Several GPATs and LPAATs have been identified from the 1-acylglycerol-3-phophate O-acyltransferase (AGPAT) family, which possess AGPAT motifs (LPLAT motifs) (13–15). Due to the fact that several groups have cloned LPLATs independently, each enzyme has multiple names. To eliminate confusion in the nomenclature, we propose that the enzymes should be renamed based on their substrate specificities and by the order in which their cloning was reported (**Fig. 2**) (7).

LPA synthesis

We will briefly summarize the acyltransferases involved in the de novo pathway. From the AGPAT family, four mammalian GPATs, which synthesize LPA from GP, have been cloned (Fig. 2). GPAT1 and GPAT2 (also called xGPAT) are located in the outer mitochondrial membrane, whereas GPAT3 (also called AGPAT8, AGPAT9, or LPAATθ) and GPAT4 (also called AGPAT6 or LPAATζ) are localized to the endoplasmic reticulum (ER). These enzymes prefer saturated and monounsaturated fatty acyl-CoAs. The microsomal GPATs are thought to play vital roles in triacylglycerol synthesis. The mitochondrial GPATs are regulated nutritionally and hormonally (12). A recent review described the cloning of GPATs (7).

PA synthesis

PA is synthesized from LPA by LPAAT. Two LPAATs (LPAAT1 and 2) have currently been cloned and characterized (Fig. 2). Human LPAAT1 (also called AGPAT1 or LPAATα) and human LPAAT2 (also called AGPAT2 or LPAATβ) were cloned based on their homologies with yeast, *E. coli*, and coconut AGPATs. Both mRNAs are found in most tissues. Human LPAAT1 shows higher activity toward 14:0-, 16:0-, and 18:2-CoAs, while human LPAAT2 prefers 20:4-CoA over 16:0- or 18:0-CoA. The AGPAT motifs have been well characterized using GPAT1, LPAAT1, and lyso-PC acyltransferase 1 (LPCAT1, described later). Taken together, these reports indicate that the AGPAT motifs contain the sequences nHxxxxD (motif 1), GxxFxxR (motif 2), EGtr (motif 3), and xxPxx (motif 4) (13–15). The amino acids in small letters in motifs 1 and 3 are not completely conserved among LPLATs and motif 4 consists of a conserved proline surrounded by hydrophobic amino acids. Site-directed mutagenesis has demonstrated that these motifs are important for LPLAT activity (13–15). LPCAT1 also has weak LPAAT activity (see "PC and PAF synthesis in

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Pathway	Product	Proposed Name	Former Name			
de novo pathway	LPA	GPAT1	GPAT1			
		GPAT2	GPAT2	xGPAT1		
		GPAT3	GPAT3	AGPAT8	AGPAT9	LPAATθ
		GPAT4		AGPAT6		LPAATζ
	PA	LPAAT1		AGPAT1		LPAATα
		LPAAT2		AGPAT2		LPAATβ
		LPCAT1	LPCAT1	AGPAT9	AT like 2	
Remodeling pathway pathway	PC	LPCAT1	LPCAT1	AGPAT9	AT like 2	
		LPCAT2	LysoPAFAT/LPCAT2		AT like 1	
		LPCAT3	MBOAT5			
		LPCAT4	MBOAT2			
		LPEAT2	LPEAT2	AGPAT7	AT like 3	LPAATη
	PE	LPCAT3	MBOAT5			
		LPCAT4	MBOAT2			
		LPEAT1	MBOAT1			
		LPEAT2	LPEAT2	AGPAT7	AT like 3	LPAATη
	PS	LPCAT3	MBOAT5			
		LPEAT1	MBOAT1			
		LPEAT2	LPEAT2	AGPAT7	AT like 3	LPAATη
	PG	LPCAT1	LPCAT1	AGPAT9	AT like 2	
		LPGAT1	LPGAT1			
		LPEAT2	LPEAT2	AGPAT7	AT like 3	LPAATη
	PI	LPIAT1	MBOA-7	MBOAT7	LRC4	
	CL	LCLAT1	ALCAT	AGPAT8		
	PAF	LPCAT1	LPCAT1	AGPAT9	AT like 2	
		LPCAT2	LysoPAFAT/LPCAT2		AT like 1	
	Unknown			AGPAT3		LPAATγ
				AGPAT4		LPAATδ
				AGPAT5		LPAATε
					AT Like 1B	

Fig. 2. A proposal for LPLAT nomenclature. Proposed names are shown in red. 1-Acylglycerol-3-phophate O-acyltransferase (AGPAT) and membrane bound O-acyltransferase (MBOAT) family members are indicated by yellow and blue, respectively. Representative former names are shown. AT like, acyltransferase like; CL, cardiolipin; GP, glycerol-3-phosphate; GPAT, acyltransferase; LCLAT, lyso-CL acyltransferase; LPA, lysophosphatidic acid; LPAAT, lyso-PA acyltransferase, LPCAT, lyso-PC acyltransferase; LPEAT, lyso-PE acyltransferase; LPGAT, lyso-PG acyltransferase; LPIAT, lyso-PI acyltransferase; PA, phosphatidic acid; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PS, phosphatidylserine.

the remodeling pathway" in later discussion). A recent review described the cloning of LPAATs (7).

Uncharacterized putative LPLATs in the AGPAT family

Three other putative LPAATs (AGPAT3, 4, and 5; also called LPAATγ, δ, and ε, respectively) have been reported as LPAATs, but their activities toward 18:1-CoA were very limited (Fig. 2) (16). Additionally, although the AT like 1B is listed as a putative mouse acyltransferase gene in the NCBI data base, no biochemical characterization has been conducted and a human homolog has not been found (Fig. 2). These gene products have not yet been analyzed in detail.

ACYLTRANSFERASES IN THE REMODELING PATHWAY (LANDS' CYCLE)

Kennedy and Weiss (8) first reported that phospholipids are synthesized in the de novo pathway (Kennedy pathway), and then Lands reported that fatty acyl composition at the *sn*-2 position is altered in the remodeling pathway (Lands' cycle) through the concerted actions of PLA_2s and LPLATs (Fig. 1) (2, 6, 7). Characterization of PLA_2s has been more extensive (2) than that of LPLATs. Recently, however, several LPLATs have been cloned and characterized as remodeling enzymes (7).

CL and PG synthesis in the remodeling pathway

CL is the only known dimeric glycerophospholipid and contains four fatty acyl linoleoyl chains (C18:2). Lyso-CL acyltransferase (LCLAT) synthesizes CL from dilyso-CL and monolyso-CL. Cao et al. (17) identified mouse LCLAT1 (also called ALCAT1 and AGPAT8) in the AGPAT family and demonstrated the diLCLAT and monoLCLAT activities of the enzyme, with a preference for 18:1-CoA and 18:2-CoA (Fig. 2). Mouse LCLAT1 is widely distributed, with the highest expression in the heart and liver. CL and PG are more abundant in the mitochondrial membranes, while LCLAT1 is localized to the ER. How PG/CL is transported from the ER to mitochondria remains to be determined, as does the complete specificity of the enzymes. CL

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remodeling is believed to play an important role in the maintenance of normal heart functions. In fact, defective CL is associated with Barth syndrome, a family disease caused by mutations of putative acyltransferase genes that manifests itself as cardiomyopathy and skeletal muscle myopathy (18).

In the de novo pathway, CL is synthesized from PG, which is also a potential activator of the protein kinase C family, including the conventional protein kinase C-β_{II} (19). PG is modified by lyso-PG acyltransferase (LPGAT) in the remodeling pathway. Human LPGAT1 was the first cloned LPGAT and shows a clear preference for 16:0, 18:0, and 18:1-CoAs as donors, consistent with the composition of endogenous PG in several tissues (Fig. 2) (20). LPGAT1 is widely distributed in tissues and is localized to the ER. On the basis of these two pioneering reports of the cloning of LCLAT1 and LPGAT1, studies of acyltransferases in the remodeling pathway have progressed rapidly.

LPCAT1 has LPCAT and LPGAT activity and primarily catalyzes the synthesis of disaturated PC and disaturated PG (Fig. 2) (21, 22). These products play an important role in lung function (see the following section for details). Lyso-PE acyltransferase (LPEAT) 2 (also called AGPAT7, LPAATη, or AT like 3) also shows LPGAT activity, but an siRNA specific for LPEAT2 could not decrease the LPGAT activity (see PE section for details) (Fig. 2) (23).

PC and PAF synthesis in the remodeling pathway

Currently, five enzymes with LPCAT activity have been identified (Fig. 2) (21–27). LPCAT1, LPCAT2, and LPEAT2 are members of the AGPAT family, whereas LPCAT3 and LPCAT4 are members of a new acyltransferase family, the membrane bound O-acyltransferase (MBOAT) family.

LPCAT1. We and Chen et al. (21, 22) independently discovered LPCAT1 (also called AGPAT9 or AT like 2), which catalyzes disaturated-PC and disaturated-PG synthesis (Fig. 2). LPCAT1 is expressed primarily in the lung, especially in alveolar type II cells, and its mRNA level was increased during the perinatal period. Disaturated phospholipids (PC > PG), mainly dipalmitoyl-PC, are major components of the pulmonary surfactant lipids, which decrease surface tension and is thereby essential for respiration. Preterm delivery causes neonatal (infantile) respiratory distress syndrome due to the lack of surfactant lipids and proteins (28). The surfactant contains small amounts of dipalmitoyl-PG and PC with linoleic acid (18:2) or linolenic acid (18:3) at the *sn*-2 position. LPCAT1 utilizes 18:2-CoA or 18:3-CoA as substrates in vitro (21). Thus, LPCAT1 may synthesize most of the phospholipids in the pulmonary surfactant and play a critical role in respiratory physiology. Further studies are needed to elucidate the direct relationship between LPCAT1 and surfactant lipid synthesis.

LPCAT2. We identified LPCAT2 (also called LysoPAFAT/LPCAT2 or AT like 1), which catalyzes both PAF and PC synthesis mainly in inflammatory cells (Fig. 2) (24). Thus, two types of glycerophospholipids (PAF and PC) are synthesized from the same precursor (lyso-PAF) using a single enzyme (LPCAT2) (24). LPCAT2 mRNA is up-regulated in mouse thioglycollate-induced peritoneal macrophages by treatment with Toll-like receptor 4 agonists, lipopolysaccharide, but the upregulation is suppressed by dexamethasone treatment. Upon acute inflammatory stimulation with lipopolysaccharide, the lyso-PAF acetyltransferase activity of LPCAT2 was activated in the p38 mitogen-activated protein kinase dependent pathway, consistent with the previous report of endogenous lyso-PAF acetyltransferase activity (29). In contrast, the LPCAT activity of LPCAT2 was not enhanced (24). Thus, a single enzyme catalyzes membrane biogenesis (LPC acyltransferase activity) of inflammatory cells, while producing PAF (lyso-PAF acetyltransferase activity) in response to external stimuli. How the two activities are differentially regulated remains to be elucidated. Furthermore, specific inhibitors of LPCAT2 may be better anti-inflammatory drugs than PAF receptor antagonists due to the fact that such inhibitors could potentially inhibit proliferation of inflammatory cells by disturbing membrane biogenesis, in addition to inhibiting the inflammatory signaling by PAF.

We recently reported that LPCAT1 also possesses a lyso-PAF acetyltransferase activity (Fig. 2) (15). In addition, the amino acid residues of LPCAT1 that are essential for each activity (LPCAT or lyso-PAF acetyltransferase activity) were identified by site-directed mutagenesis. In contrast to LPCAT2, LPCAT1 was neither activated nor up-regulated by lipopolysaccharide stimulation in the mouse macrophages. While LPCAT2 activity is Ca^{2+}-dependent, LPCAT1 activity is Ca^{2+}-independent. These findings indicate two distinct remodeling pathways for PAF synthesis: the inflammatory/inducible (LPCAT2) and the noninflammatory/constitutive (LPCAT1) remodeling pathway (15). The relationship between LPCAT1 and LPCAT2 is similar to that of cyclooxygenase 1 and 2, which are constitutively expressed and inducible enzymes, respectively (30). It is still possible that other constitutive lyso-PAF acetyltransferase is present, as LPCAT1 is enriched only in alveolar type II cells.

LPEAT2 also shows LPCAT activity, but an siRNA specific for LPEAT2 did not decrease the LPCAT activity (see PE section for detail) (Fig. 2) (23). In addition, LPEAT2 (AGPAT7) was reported to exhibit LPCAT activity in red blood cells, although the activity was relatively weak (31).

LPCAT3 and LPCAT4. We and Zhao et al. (25, 26) independently identified LPCAT3 in the MBOAT family, which contains diacylglycerol acyltransferase 1; cholesterol acyltransferases 1 and 2; and protein acyltransferases; such as ghrelin acyltransferase (2, 7, 32). LPCAT3 (also called MBOAT5), exhibits LPCAT, LPEAT, and lyso-PS acyltransferase (LPSAT) activities (Fig. 2) (25–27). Thus, the MBOAT family is a novel LPLAT family. Mouse LPCAT3 mRNA was detected ubiquitously and showed higher acyltransferase activity toward polyunsaturated fatty acyl-CoAs, 20:4-CoA and 18:2-CoA, than saturated fatty acyl-CoAs. Transfection with LPCAT3-siRNA into B16 melanoma cells reduced endogenous LPCAT, LPEAT, and LPSAT activities with 20:4-CoA and the amount of PC, PE, and PS containing arachidonic acid at the *sn*-2 position.

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LPCAT4 (originally called MBOAT2) possesses LPCAT and LPEAT activities with a clear preference for 18:1-CoA (Fig. 2) (25). Mouse LPCAT4 mRNA is highly expressed in the epididymis, brain, testis, and ovary. Because LPCAT3 and LPCAT4 had higher LPCAT activity with 1-acyl-LPC than 1-*O*-alkyl-LPC or 1-*O*-alkenyl-LPC as acceptors, these enzymes may recognize the difference between the ester and ether bond at the *sn*-1 position of LPC (25).

PE and PS synthesis in the remodeling pathway

LPEAT1 (also called MBOAT1) is a member of the MBOAT family and possesses LPEAT and LPSAT activities with a clear preference for 18:1-CoA (Fig. 2) (25). Mouse LPEAT1 mRNA is highly expressed in the stomach, epididymis, and colon. LPCAT3 and LPCAT4 also have LPEAT activities toward polyunsaturated acyl-CoAs and 18:1-CoA, respectively. In contrast to the LPCAT activities of LPCAT3 and LPCAT4, no clear differences were observed between the 1-acyl-LPEAT and 1-*O*-alkenyl-LPEAT activities of each enzyme. LPCAT3 and LPEAT1 catalyze PS synthesis in the remodeling pathway (Fig. 2). LPCAT3 recognizes polyunsaturated acyl-CoAs and LPEAT1 prefers 18:1-CoA in the presence of LPS. Thus, LPCAT3 synthesizes PC, PE, and PS containing polyunsaturated fatty acids at the *sn*-2 position. In contrast, LPCAT4 (PC and PE formation) and LPEAT1 (PE and PS formation) esterify oleic acid (18:1) to the *sn*-2 position of lysophospholipids (25).

Recently, LPEAT2 (previously called AGPAT7, LPAATη, or AT like 3) was identified from the AGPAT family (23). The enzyme is similar to LPCAT1 and LPCAT2, and show LPEAT, LPGAT, LPSAT, and LPCAT activities toward 18:1-CoA or 20:4-CoA (Fig. 2). However, an siRNA specific for LPEAT2 decreased only the LPEAT activity, but not the LPGAT, LPSAT, or LPCAT activities in HEK293T cells (23). Due to the fact that mouse LPEAT2 was expressed primarily in the brain, LPEAT2 may play crucial roles in the biogenesis of brain PE.

PI synthesis in the remodeling pathway

Lyso-PI acyltransferase (LPIAT)1 (also called MBOA-7, MBOAT7, and LRC4) is the first PI synthetic enzyme in the remodeling pathway to be identified (33) (Fig. 2). LPIAT1 exhibits LPIAT activity toward 20:4-CoA and the site-directed mutagenesis of human LPIAT1 revealed a predicted active site residue, His350 within a long hydrophobic region. An LPIAT1 mutant of *C. elegans* exhibits larval arrest and egg-laying defects. Mammals may possess more than one LPIAT.

CONCLUSION

Phospholipids are the major constituents of biological membranes, playing important roles in the maintenance of the cellular boundary and the regulation of cellular signaling. Kennedy and Lands reported the synthesis of phospholipids in the de novo pathway (Kennedy pathway), and the maturation of phospholipids in the remodeling pathway (Lands' cycle), respectively, to produce membrane diversity (Fig. 1). Membrane diversity is important for membrane fluidity and curvature and is produced by the concerted and overlapping reactions of multiple LPLATs that recognize both the polar head groups of lyso-glycerophospholipids and various acyl-CoAs in the remodeling pathway. In the last 4 years, many LPLATs functioning in the remodeling pathway have been identified, resulting in the most spectacular advance in the LPLAT field since the discovery of the Kennedy pathway and the Lands' cycle 50 years ago (2, 7). As each enzyme has several names and each name refers to several enzymes, we have proposed renaming LPLATs in order to clarify and standardize the nomenclature (see Fig. 2) (7). Identification of additional LPLATs may contribute to further elucidation of membrane diversity and asymmetry. It will be intriguing to determine how many enzymes are present and sufficient to produce over 800 different molecular species of glycerophospholipids.

Because acyl-CoAs and lysophospholipids are the substrates of LPLATs, clarification of which acyl-CoA synthetases (an alternative name; acyl-CoA ligase) and PLA_2s are functionally coupled to individual LPLATs will be important. How phospholipids synthesized in the ER are transported to target organelles, including lamellae bodies in alveolar type II cells, remains to be determined. Additionally, analysis of enzyme activities with mixtures of acyl-CoA or lysophospholipid substrates is important. Although enzyme purification is not an easy task because of the multi-transmembrane spanning nature of the LPLATs, determination of the 3D structure by X-ray crystallography is of interest. Further studies are needed to elucidate the biological roles of these enzymes in vivo, such as analyzing LPLAT knockout mice or in vivo siRNA experiments. The recent findings reviewed here constitute a critical milestone for greater understanding of how membrane diversity and asymmetry are established and the biological significance of these phenomena.

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