厚生労働科学研究費補助金

免疫アレルギー疾患等予防・治療研究事業

新規生体膜生合成酵素と生理活性脂質（PAF）生合成
酵素の機能解析
（H20-免疫-若手-028）

平成20年度　総括研究報告書

研究代表者　進藤　英雄

平成21（2009）年　4月

目　　次

新規生体膜生合成酵素と生理活性脂質（PAF）生合成酵素の機能解析

研究代表者　進藤　英雄　　東京大学大学院医学系研究科　助教

全ての生き物は細胞から成り、その細胞は生体膜に覆われている。その生体膜の主成分はリン脂質であり、組織によって多種多様である。1950年代に、この多様なリン脂質はリゾリン脂質アシル転移酵素によって形成されることが報告され、その生合成経路はランズ回路と呼ばれた。それから50年間その遺伝子群は不明であったが、近年私達はリゾリン脂質アシル転移酵素ファミリーを発見した。また、強力な免疫アレルギーのメディエーターである血小板活性化因子（PAF）もリン脂質であり、その生合成酵素も私達は初めて同定し、LPCAT2と名付けた。LPCAT2もリゾリン脂質アシル転移酵素である。これら新規に同定されたリン脂質生合成酵素を解析し、マクロファージなどの炎症細胞における生体膜生合成やPAF生合成メカニズムの解明を進め、炎症免疫に強い細胞環境（生体膜環境）を解明する。

A．研究目的

生体膜の主成分であるリン脂質は組織や細胞によって多種多様であり、血小板活性化因子(PAF)のような炎症性物質も含まれる。PAFは炎症や免疫を惹起する強力なメディエーターである。この生体膜の多様性は、リン脂質から脂肪酸を遊離させるホスホリパーゼA_2（PLA_2）と脂肪酸を再結合させるリゾリン脂質アシル転移酵素によるリン脂質の代謝回転によって作られ、ランズ回路と名付けられている。これまでPLA_2は複数の分子同定が進み、よく研究されてきたが、一方のリゾリン脂質アシル転移酵素は膜結合型の酵素であることから、ほとんど分子同定されず、ランズ回路発見以来50年間進展が難しかった。しかし、生体膜の多様性は、このリゾリン脂質アシル転移酵素によって決められる。私達はゲノムデータベースを利用し候補遺伝子を約20種類クローニングし、

PAF生合成酵素を含む、リン脂質生合成酵素（リゾリン脂質アシル転移酵素）を5種類同定した。このうち3種類は新たなアシル転移酵素遺伝子ファミリーを形成していた。中にはPAF生合成酵素であるリゾPAFアセチル転移活性を持つ分子も含まれており、リポポリサッカライド（LPS）刺激で活性化や遺伝子誘導されることもわかった。これらの酵素群の機能解析を行うことによって、マクロファージなどの炎症細胞での生体膜形成メカニズムやPAF産生メカニズムを解明する。そして、免疫、アレルギーに強い細胞環境（膜）の解明やPAF関連疾患の治療を目指す。

B．研究方法

リゾリン脂質アシル転移酵素はリゾリン脂質にアシルCoAから脂肪酸を転移する活性を持つ。例えばリゾホスフ

ァチジルコリンアシル転移酵素
（LPCAT）はリゾホスファチジルコリ
ン（LPC）からホスファチジルコリン
（PC）を生合成する酵素である。これ
まで、我々が同定したリゾリン脂質ア
シル転移酵素は、（i）肺に多く呼吸
に必須な肺サーファクタント脂質を
合成するLPCAT1と（ii）PAF生合成酵
素であるLPCAT2、さらに、新規に発見
した遺伝子ファミリーからは恒常的
に生体膜を合成すると考えられる酵
素（iii）LPCAT3、（iv）LPCAT4、（v）
リゾホスファチジルエタノールアミ
ンアシル転移酵素1（LPEAT1）である。
これら5分子を中心に、全てのリゾリ
ン脂質アシル転移酵素をターゲット
として研究を進めている。さらに今回
（1）2番目のPAF生合成酵素と（2）
新規にリゾホスファチジン酸アシル
転移酵素3(LPAAT3)を同定した。

1年目として以下の実験を行った
（1-1）2番目のPAF生合成酵素（リ
ゾPAFアセチル転移酵素）の同定
これまで、PAF生合成酵素（リゾPAFア
セチル転移酵素）は反応にCaを要求す
ることが酵素遺伝子を特定しない実
験（組織抽出タンパク質）から報告さ
れていた。実際に我々が同定した
LPCAT2はCa要求性のPAF生合成酵素で
あった。しかし、今回Ca非要求性のPAF
生合成酵素活性を主にマウス肺で見
つけた。肺のミクロソーム画分を界面
活性剤であるBIGCHAPで可溶化し陰イ
オン交換カラムであるDEAEセファロ
ースカラム(GE)で部分精製した。分離
にはAKTA explorer 10S(GE)を用いた。

（1-2）LPCAT1の酵素学的解析
LPCAT1の基質（アシルCoA）結合部位
や活性中心の推定を目的とし、保存性
の高いモチーフを中心に詳細な点変
異解析を行った。また、マウスマクロ

ファージにおいてtoll-like受容体4
（TLR4）、TLR3、TLR9アゴニストによ
る自然免疫刺激が酵素の活性や遺伝
子発現に与える影響を調べた。

（2）新規リゾリン脂質アシル転移酵
素の発見
既知のアシル転移酵素モチーフを基
に候補遺伝子が複数見つけられてい
る。その中から
1-acylglycerol-3-phosphate
O-acyltransferase 3（AGPAT3）に注目
し、網羅的にアシル転移酵素活性測定
を行った。さらにAGPAT3の高発現組織
やmRNA発現誘導などを調べた。

酵素活性測定は放射ラベルされた基
質を用いて反応し、Bligh-Dyer法で反
応産物であるリン脂質を抽出し、薄層
クロマトグラフィー（TLC）で分離後、
放射活性をカウントした。遺伝子変動
はtotal RNAを抽出し定量的PCRを行い
解析した。

C．研究結果
（1-1）2番目のPAF生合成酵素（リ
ゾPAFアセチル転移酵素）の同定
マウス肺のミクロソーム画分から
AKTA explorer 10Sを用いて部分精製
した。陰イオン交換カラムであるDEAE
セファロースカラムに酵素は吸着し、
NaClにより容出された。既知のPAF生
合成酵素であるLPCAT2と最も相同性
が高く肺に強く発現するアシル転移
酵素はLPCAT1であったため、各画分を
抗LPCAT1抗血清を用いて発現を調べ
ると酵素活性と抗LPCAT1抗血清によ
る検出パターンがほぼ一致した。そこ
で生化学的にLPCAT1の基質特異性を
詳しく調べると、LPCAT1もPAF生合成
酵素活性をもつことがわかった。この
活性はLPCAT2と異なり、Ca非要求性で

2

あった。また、MgやMnも酵素活性に必要としなかった。

（1-2）LPCAT1の酵素学的解析
今回、LPCAT1は2種類の活性を持つことがわかった。一つは肺サーファクタント脂質合成活性（アシル転移活性；PC生合成）、もう一つはPAF生合成活性（アセチル転移活性；炎症メディエーター）。それぞれの活性に必要なアミノ酸も点変異解析により同定した。特に、Ile160はアシル転移活性に、Phe174、Val175、Arg177はアセチル転移活性必須であった。Arg177はCoAと結合すると予測できた。
さらに、マウスチオグリコレートマクロファージをTLR4、TLR3、TLR9アゴニストで長時間（16時間程度）刺激するとLPCAT2の発現が約9倍に上昇したがLPCAT1は誘導されなかった。また、短時間刺激（30分程度）でもLPCAT2が活性化されたが、LPCAT1活性は変動しなかった。

（2）新規リゾリン脂質アシル転移酵素の発見
AGPAT3はリゾホスファチジン酸アシル転移酵素（LPAAT）活性とリゾホスファチジルイノシトールアシル転移酵素（LPIAT）活性をアラキドノイルCoA（20:4-CoA）をドナーとして示した。これはグリセロール骨格sn-2位にアラキドン酸を持つホスファチジン酸（PA）とホスファチジルイノシトール（PI）を合成することになる。酵素活性からAGPAT3をLPAAT3に改名した。アシル転移酵素モチーフの一部をAlaに置換した変異体では両活性が認められなかった。マウス組織でのmRNA発現は主に精巣であった。さらにマウス精巣において週齢に応じてLPAAT3の発現量が上昇した。さらに精巣培養細胞TM4をエストラジオール17βで刺激す

るとLPAAT3 mRNA発現量が4倍に上昇した。

D．考察
（1）2番目のPAF生合成酵素；LPCAT1の解析
LPCAT1とLPCAT2の解析から、発現場所が限局されているが、LPCAT1（主に肺）は恒常的に働く酵素で、LPCAT2（主にマクロファージ）は誘導型のPAF生合成酵素であることがわかった。これらはアラキドン酸からプロスタグランジンH2を合成するシクロオキシゲナーゼ（COX）-1（恒常型）とCOX-2（誘導型）の関係に似ている。これまで、PAFの生合成経路は2種類（de novo経路とリモデリング経路）報告されており、リゾPAFアセチル転移酵素はリモデリング経路の酵素である。定常時のPAF量はde novo経路で維持され、炎症時はリモデリング経路で産生されると考えられてきたが、LPCAT1については定常時にリモデリング経路でPAFを産生することがわかり、新しいPAF合成経路の発見となった。今後はLPCAT1とLPCAT2の生体内での誘導やPAF産生における役割分担、さらにPAFによるアレルギーメカニズムの解明が必要になる。また、細菌感染時おける、生体膜変化やPAFの役割解明も必要となる。

（2）新規リゾリン脂質アシル転移酵素の発見
LPAAT3はPAとPIを生合成する活性を持った。PAはPIの前駆体にもなりうるためLPAAT3は2種類のステップを触媒してアラキドン酸を含有するPIを効率よく合成していることになる。また、マウス精巣においてLPAAT3のmRNA発現量は週齢と相関して上昇し、さらに精巣培養細胞TM4でもエストラジオール17β刺激で発現上昇した。ヒト男性

3

でも年齢とともにエストラジオール量が増加することは報告されている。LPAAT3の発現はエストラジオールによって調節され、精巣の成熟と関連しているかもしれない。また、グリセロール骨格sn-2位にアラキドン酸を持つリン脂質を増やすことは、アラキドン酸由来の生理活性脂質（ロイコトリエン、プロスタグランジンなど）の産生にも寄与しているであろう。

E．結論

本研究では生体膜生合成酵素とそれに類似したPAF生合成酵素の機能解析から、炎症性細胞等の感染時における生体膜変化の解明を目的としている。一年目は、恒常的に働く新規PAF生合成酵素の発見や、活性中心の同定等に成功した。また、PAF生合成酵素の自然免疫応答による誘導の解明も進んだ。2種類のPAF生合成酵素（恒常型LPCAT1と誘導型LPCAT2）のそれぞれに特異的な阻害剤などの開発が望まれる。PAFの生合成メカニズムの解明は炎症やアレルギーの新しい治療方法の開発につなげられるであろう。

また、今回新たに発見したLPAAT3は精巣の成熟に関係する可能性がわかった。生理活性脂質の貯蔵の役割も考えられる。今後は細胞やマウス、ヒトレベルでの解析を進める。酵素欠損細胞だけでなく、作製が進んでいる複数種類の酵素欠損マウスの解析を行うことにより、より臨床応用できる成果を目指す。

F．健康危険情報
なし

G．研究発表
1．論文発表

(1) Yuki, K., **Shindou, H**., Hishikawa, D., and Shimizu, T. (2009) Characterization of mouse lysophosphatidic acid acyltransferase 3: An enzyme with dual functions in the testis. J. Lipid Res., in press

(2) **Shindou H.,** Hishikawa D., Harayama T., Yuki K., and Shimizu T. (2009) Recent progress on Acyl-CoA:lysophospholipid acyltransferase research. J. Lipid Res. In press

(3) **Shindou H.** and Shimizu T. (2009) Acyl-CoA:lysophospholipid acyltransferases. J. Biol. Chem. 284, 1-5

(4) Kihara, Y., Yanagida, K., Masago, K., Kita, Y., Hishikawa, D., **Shindou, H.,** Ishii, S., and Shimizu, T. (2008) Platelet-Activating Factor Production in the Spinal Cord of Experimental Allergic Encephalomyelitis Mice via the Group IVA Cytosolic PLA2-LysoPAFAT Axis. J. Immunol. 181, 5008-14

(5) Harayama, T*., **Shindou, H*. (*, equal contribution)**, Ogasawara, R., Suwabe, A, and Shimizu, T. (2008) Identification of a novel non-inflammatory biosynthetic pathway of platelet-activating factor. J. Biol. Chem. 283, 11097-11106

(6) Tsujimura, Y., Obata, K., Mukai, K., **Shindou, H.**, Yoshida, M., Nishikado, H., Kawano, Y., Minegishi, Y., Shimizu, T. and Karasuyama, H . (2008) Basophils play a pivotal role in IgG- but not IgE-mediated systemic anaphylaxis in

4

contrast to mast cells. Immunity 28, 581-9.

342

2. 学会発表
（発表誌名巻号・頁・発行年等も記入）

国内学会
(1) **進藤英雄**、菱川大介、清水孝雄（東大・院医・細胞情報）リゾリン脂質アシル転移酵素LPEAT1とLPCAT4の機能解析　日本分子生物学会年会日本生化学会大会合同大会　神戸2008年12月9-12日　BMB2008 Abstracts P. 338

(2) 菱川大介、**進藤英雄**、清水孝雄（東大・院医・細胞情報）リゾリン脂質アシル転移酵素LPCAT3の機能解析　日本分子生物学会年会日本生化学会大会合同大会　神戸2008年12月9-12日　BMB2008 Abstracts P. 338

(3) 原山武士、**進藤英雄**、清水孝雄（東大・院医・細胞情報）ヒトLPCAT1のクローニングと解析　日本分子生物学会年会日本生化学会大会合同大会　神戸2008年12月9-12日　BMB2008 Abstracts P. 338

(4) 清水孝雄、**進藤英雄**（東大・院医・細胞情報）生体膜リン脂質の多様性と非対称生を決める酵素ファミリー　日本分子生物学会年会日本生化学会大会合同大会　神戸2008年12月9-12日　BMB2008 Abstracts P. 66

(5) 木原泰行、石井聡、**進藤英雄**、北芳博、清水孝雄「多発性硬化症モデルマウスにおける血小板活性化因子の代謝機構」日本分子生物学会年会日本生化学会大会合同大会　神戸2008年12月9-12日　BMB2008 Abstracts P.

(6) **進藤英雄**1、菱川大介1、小林早織1、中西広樹2、田口良2、清水孝雄1（1東大・院医・細胞情報）（2東大・院医・メタボローム寄附講座）新規リゾリン脂質アシル転移酵素LPEAT1とLPCAT4の同定と解析
脂質生化学会　2008　6/5-6　徳島
『脂質生化学研究』、日本脂質生化学会、50号、pp. 191-194　2008

(7) 菱川大介1、**進藤英雄**1、小林早織1、中西広樹2、田口良2、清水孝雄1（1東大・院医・細胞情報）（2東大・院医・メタボローム寄附講座）新規リゾリン脂質アシル転移酵素LPEAT1とLPCAT4の同定と解析
脂質生化学会　2008　6/5-6　徳島
『脂質生化学研究』、日本脂質生化学会、50号、pp. 195-197　2008

(8) 原山武士1、**進藤英雄**1、小笠原理恵2、諏訪部章2、清水孝雄1
1東大・院医・細胞情報
2岩手医大・医・臨床検査医学
リゾホスファチジルコリンアシル基転移酵素LPCAT1は非炎症時において血小板活性化因子を合成する
脂質生化学会　2008　6/5-6　徳島
『脂質生化学研究』、日本脂質生化学会、50号、pp. 198-200　2008

(9) **進藤英雄**、原山武士、清水孝雄
題名：II型肺胞上皮細胞における非炎症性PAF生合成酵素の同定
札幌医科大学記念ホール　2008年7月5日（土）
分子呼吸器病、先端医学社、(2009) Vol. 13 No. 1. 120-124

国際学会
(1) **Hideo Shindou**, Hishikawa Daisuke,

and Takao Shimizu. Identification of lysophospholipid acyltransferases, LPEAT1 and LPCAT4. FASEB Phospholipid Metabolism: disease, signal transduction and membrane dynamics. July 20-25, 2008 **Oral Presentation** New Haven, Connecticut

(2) Hishikawa Daisuke, **Hideo Shindou**, and Takao Shimizu. Identification of lysophospholipid acyltransferases, LPCAT3. FASEB Phospholipid Metabolism: disease, signal transduction and membrane dynamics. July 20-25, 2008
New Haven, Connecticut

(3) Takao Shimizu and **Hideo Shindou**. Lysophosphatidylcholne acyltransferase 1 and 2. FASEB Phospholipid Metabolism: disease, signal transduction and membrane dynamics. July 20-25, 2008
New Haven, Connecticut

H．知的財産権の出願・登録状況
なし

III. 研究成果の刊行に関する一覧表
　雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Shindou H. and Shimizu T.	Acyl-CoA:lysophospholipid acyltransferases.	J. Biol. Chem.	284	1-5	2009
Shindou H., Hishikawa D., Harayama T., Yuki K., and Shimizu T.	Recent progress on Acyl-CoA:lysophospholipid acyltransferase research.	J. Lipid Res.		In press	2009
Yuki, K., **Shindou, H.**, Hishikawa, D., and Shimizu, T.	Characterization of mouse lysophosphatidic acid acyltransferase 3: An enzyme with dual functions in the testis.	J. Lipid Res.		In press	2009

MINIREVIEW

Acyl-CoA:Lysophospholipid Acyltransferases[*]

Hideo Shindou and Takao Shimizu[1]

From the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Cell membranes contain several classes of glycerophospholipids, which have numerous structural and functional roles in the cells. Polyunsaturated fatty acids, including arachidonic acid and eicosapentaenoic acid, are located at the sn-2 (but not sn-1)-position of glycerophospholipids in an asymmetrical manner. Using acyl-CoAs as donors, glycerophospholipids are formed by a *de novo* pathway (Kennedy pathway) and modified by a remodeling pathway (Lands' cycle) to generate membrane asymmetry and diversity. Both pathways were reported in the 1950s. Whereas enzymes involved in the Kennedy pathway have been well characterized, including enzymes in the 1-acylglycerol-3-phosphate *O*-acyltransferase family, little is known about enzymes involved in the Lands' cycle. Recently, several laboratories, including ours, isolated enzymes working in the remodeling pathway. These enzymes were discovered not only in the 1-acylglycerol-3-phosphate *O*-acyltransferase family but also in the membrane-bound *O*-acyltransferase family. In this review, we summarize recent studies on cloning and characterization of lysophospholipid acyltransferases that contribute to membrane asymmetry and diversity.

Biosynthetic Pathway

All organisms are composed of cells that are enclosed by a cell membrane, which contains phospholipids, cholesterol, and proteins. Lipids fulfill four general functions. (i) They serve as an efficient source of energy; (ii) they form cell membranes that contain the bipolar lipids of glycerophospholipids and sphingophospholipids; (iii) they participate in the regulation of particular proteins through post-translational lipid modification; and (iv) they serve as messengers during cellular signal transduction (1). Thus, glycerophospholipids are important not only as structural and functional components of cell membranes but also as precursors of various lipid mediators, such as PAF[2] and

eicosanoids (2, 3). Each tissue maintains a distinct content and composition of various phospholipids, such as PA, PC, PE, PG, CL, PI, and PS (1, 4, 5). For the biosynthesis of glycerophospholipids, fatty acids first need to be activated to acyl-CoAs as described by Kornberg and Pricer (6). Using acyl-CoAs as donors, phospholipids are formed from glycerol 3-phosphate by the *de novo* pathway, originally described by Kennedy and Weiss in 1956 (7). However, the acyl groups of glycerophospholipids are highly diverse and are distributed in an asymmetric manner (4, 8). Saturated and monounsaturated fatty acids are usually esterified at the sn-1-position, whereas polyunsaturated acyl groups are esterified at the sn-2-position. This diversity and asymmetry is not fully explained by the Kennedy pathway. Rapid turnover of the sn-2-acyl moiety of glycerophospholipids was originally described by Lands as the remodeling pathway (Lands' cycle) (9) and is attributed to the concerted and coordinated actions of PLA$_2$s and LPLATs (3, 8, 10). Although these metabolic processes occur in a variety of tissues, information on the enzymes involved in phospholipid remodeling has been lacking for the past 50 years. Dr. Lands asked in his review, "Which enzymes distinguish between saturated and unsaturated acyl chains?" (8). Now, we may be able to answer that question because several LPLATs have been recently cloned and characterized. In this review, after a brief description of the enzymes of the Kennedy pathway, we will summarize recent findings on the cloning and characterization of remodeling enzymes in the Lands' cycle.

Acyltransferases in the *de Novo* Pathway (Kennedy Pathway)

In the *de novo* pathway of glycerophospholipid biosynthesis, LPA is first formed from glycerol 3-phosphate by GPAT (11, 12). Next, LPA is converted to PA by LPAATs, and PA is metabolized into two types of glycerol derivatives (11, 12). One is DAG, which is then converted to TAG, PC, and PE. Subsequently, PS is synthesized from PC or PE. The other glycerol derivative is cytidine diphospho-DAG, which is transformed into PI, PS, PG, and CL (Fig. 1). Several key enzymes in the *de novo* pathways have been characterized, and additional information is available in other review articles (1, 11, 12).

Several acyltransferases that form LPA or PA have been identified, and all of them are members of the AGPAT family, which possesses LPLAT motifs (13, 14). Because several groups independently cloned LPLATs, they have multiple names. For example, AGPAT1 is also called LPAATα, and AGPAT8 is also known as AGPAT9, LPAATθ, or GPAT3. Confusingly, LCLAT1 was also given the name AGPAT8. To eliminate the

confusion about the nomenclature, we propose that the enzymes be renamed based on their substrate specificities and by the order of their cloning publications (Table 1).

GPATs—Four mammalian GPATs have been cloned (15–19). GPAT1 and GPAT2 (also called xGPAT (16)) are located in the outer mitochondrial membrane, whereas GPAT3 (called AGPAT8, AGPAT9, or LPAATθ) and GPAT4 (called AGPAT6 or LPAATζ) are localized to the ER. GPAT1 is resistant to sulf-hydryl agents like *N*-ethylmaleimide and prefers 16:0-CoA as a substrate (12). In the liver of GPAT1 knock-out mice, the palmitate (16:0) content was lower at the *sn*-1-position of TAG, PC, and PE, indicating the important role of the mitochondrial form of GPAT in TAG and glycerophospholipid formation (12). GPAT2 is *N*-ethylmaleimide-sensitive, has no preference for 16:0-CoA, and is expressed mainly in mouse testis (20). The

microsomal form of GPAT constitutes ~90% of the total GPAT activity in most tissues but only 50–80% of the activity in the liver (12). Moreover, in differentiating 3T3-L1 adipocytes, the specific activity of microsomal GPAT is 70-fold higher, whereas mitochondrial GPAT activity is only 10-fold higher (12). The mRNA level of GPAT3 is consistently 60-fold higher in 3T3-L1 adipocytes than in preadipocytes (17). On the other hand, GPAT4 is expressed in many tissues. Both GPAT3 and GPAT4 recognize a broad range of substrates from 12:0-CoA to 18:1- or 18:2-CoA as donors. The microsomal form of GPAT is thought to play vital roles in TAG synthesis. The mitochondrial form of GPAT is regulated nutritionally and hormonally (12).

LPAATs—GPATs catalyze the formation of LPA from glycerol 3-phosphate, and LPAATs subsequently catalyze the formation of PA from LPA in the *de novo* pathway. To date, two LPAATs (LPAAT1 and LPAAT2) have been cloned and characterized (21–24), and three additional LPAAT candidates (AGPAT3–5) have been reported but have not been analyzed in any detail (25). They are all members of the AGPAT family and have four LPLAT motifs. Human LPAAT1 (called AGPAT1 or LPAATα) and human LPAAT2 (called AGPAT2 or LPAATβ) were cloned based on their homologies to yeast, *Escherichia coli*, and coconut AGPATs (21, 22, 24). Human LPAAT1 is expressed ubiquitously (21, 22). LPAAT1 showed higher activity with 14:0-, 16:0-, and 18:2-CoAs and showed intermediate activities with 18:1- and 20:4-CoAs (26). The LPLAT motifs of human LPAAT1 contain the sequences NHX$_4$D (motif I, residues 103–109), GVIFIDR (motif II, residues 143–149), EGTR (motif III, residues 178–181), and IVPIVM (motif IV, residues 205–210) (14). Site-directed mutagenesis of LPAAT1 demonstrated that these motifs are essential for LPAAT activity (14).

Human LPAAT2 mRNA is found in most tissues, with the highest expression seen in the heart, liver, and adipocytes (21, 24, 27). LPAAT2 prefers 20:4-CoA rather than 16:0- or 18:0-CoA (24, 26). Mutations in LPAAT2 have been linked to congenital generalized lipodystrophy (also known as Berardinelli-Seip syndrome) (27), indicating that LPAAT2 is involved in TAG synthesis and storage in adipocytes.
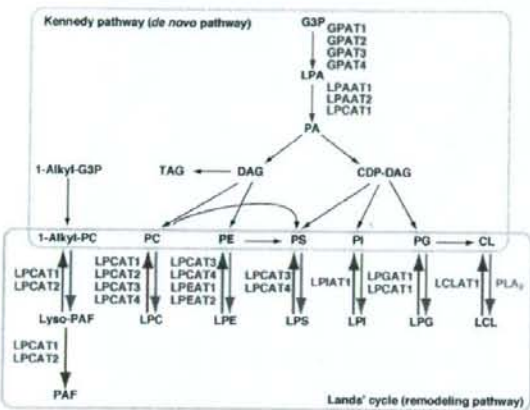

FIGURE 1. **Pathways of glycerophospholipid biosynthesis.** Glycerophospholipids are first synthesized through the *de novo* pathway (Kennedy pathway) and then modified through the remodeling pathway (Lands' cycle). *Red and blue arrows* indicate acyltransferases and PLA$_2$s, respectively. See text for detail. *G3P*, glycerol 3-phosphate; *LPC*, *LPE*, *LPS*, *LPI*, *LPG*, and *LCL*, lyso-PC, lyso-PE, lyso-PS, lyso-PI, lyso-PG, and lyso-CL, respectively; *CDP-DAG*, cytidine diphospho-DAG.

TABLE 1

Summary of LPLATs: a proposal for the standardization of LPLAT nomenclature

We propose to rename LPLATs as indicated below and discussed in text. The weaker activities with some acyl-CoAs have been omitted. The accession number for each enzyme is available in the DDBJ/GenBank™/EBI Data Bank.

Pathway	Family	Proposed Name	Former Name				Activity	Acyl-CoA Selectivity	Mouse	Human	Note.	Ref No.
de novo (Kennedy)	AGPAT	GPAT1	GPAT1				GPAT	16:0	NP_032175	NP_065969		15
		GPAT2	GPAT2	xGPAT1			GPAT	16:0	NP_001074558	NP_992211		16, 20
		GPAT3	GPAT3	AGPAT8	AGPAT9	LPAATθ	GPAT	12:0, 16:0, 18:1, 18:2	NP_766303	NP_116106		17
		GPAT4		AGPAT6	LPAATζ		GPAT	12:0, 16:0	NP_061213	NP_848934		18, 19
		LPAAT1		AGPAT1	LPAATα		LPAAT	14:0, 16:0, 18:2	NP_061350	AAH96378		21, 22, 23, 26
		LPAAT2		AGPAT2	LPAATβ		LPAAT	16:0, 18:0, 18:1, 18:2, 20:4	NP_080488	AAC51649		21, 24, 26
remodeling (Lands)		LPGAT1	LPGAT1				LPGAT	16:0, 18:0, 18:1	NP_758470	NP_055688		33
		LCLAT1	ALCAT	AGPAT8			LCLAT	18:1, 18:2,	Q3UN02	NP_001074540		28, 30
		LPCAT1	LPCAT1	AGPAT9		AT like 2	LPCAT	2:0, 6:0-16:0, 18:2, 18:3	BAE94687	BAE94688	lyso-PAF acetyltransferase	34, 35, 40, 43
							LPAAT	16:0				
							LPGAT	16:0				
		LPCAT2	LysoPAFAT/LPCAT2			AT like 1	LPCAT	2:0, 20:4	BAF47695	BAF47696	lyso-PAF acetyltransferase	37, 43
		LPEAT2	LPEAT2	AGPAT7	LPAATη	AT like 3	LPEAT	16:0, 18:0, 18:1, 20:4	NP_997089	NP_705841		42, 43
				AGPAT3	LPAATγ		unknown		NP_443747	NP_001032642		25
				AGPAT4	LPAATδ		unknown		NP_080920	NP_064518		25
				AGPAT5	LPAATε		unknown		NP_081068	NP_060831		25
						AT Like 1B	unknown		NP_081875			-
	MBOAT	LPCAT3	LPCAT3	MBOAT5			LPCAT	18:1, 18:2, 20:4	BAG12120	NP_005759		54, 55
							LPEAT	18:1, 18:2, 20:4				
							LPSAT	18:1, 18:2, 20:4				
		LPCAT4	LPCAT4	MBOAT2			LPCAT	18:1	BAG12122	NP_620154		54
							LPEAT	18:1				
		LPEAT1	LPEAT1	MBOAT1			LPEAT	18:1	BAG12121	NP_001073949		54
							LPSAT	18:1				
		LPIAT1	MBOA-7	MBOAT7	LRC4		LPIAT	20:4	NP_084210	ABV66273		56

9

Putative LPLATs in the AGPAT Family—Three other putative LPAATs (AGPAT3–5; also called LPAATγ, LPAATδ, and LPAATε, respectively) have been reported, but their LPAAT activity with 18:1-CoA was very low (25). Additionally, a putative mouse acyltransferase gene, AT-like 1B, is registered in the NCBI Database; however, the biochemical characteristics were not provided, and a human homolog has not been found.

Acyltransferases in the Remodeling Pathway (Lands' Cycle)

Phospholipids are first synthesized in the *de novo* pathway, and their fatty acyl composition at the *sn*-2-position is altered in the remodeling pathway (Lands' cycle) through the concerted actions of PLA$_2$s and LPLATs (9). Although several PLA$_2$s have been identified and well characterized (3), the cloning and characterization of acyltransferases are only occurring now. LPLATs were recently shown to be remodeling enzymes, and their biochemical analyses are in progress.

LCLAT1 and LPGAT1—CL is the only known dimeric glycerophospholipid and consists of four fatty acyl chains, a linoleoyl group (C18:2). CL is required for the reconstituted activity of several key mitochondrial enzymes involved in energy metabolism (5). CL is synthesized from dilyso-CL and monolyso-CL by LCLAT. Mouse LCLAT1 (called ALCAT1 by Cao *et al.*) was identified and found to possess LPLAT motifs (28). Overexpression of LCLAT1 in Sf9 cells or COS-7 cells led to a significant increase in di-LCLAT and mono-LCLAT activities. The enzyme recognized both dilyso-CL and monolyso-CL as acceptors with a preference for 18:1- and 18:2-CoAs. Mouse LCLAT1 is widely distributed, with the highest expression seen in the heart and liver. When LCLAT1 was overexpressed in COS-7 cells, the enzyme localized to the ER. The subcellular localization was predicted by the existence of a KK*XX* motif, an ER retention signal at the C-terminal end (29) of LCLAT1. Although another AGPAT8 (not GPAT3) was reported as being an LCLAT, its biochemical characteristics were not analyzed in detail (30). CL remodeling is believed to play an important role in the maintenance of normal heart functions. Defective CL is associated with Barth syndrome, a family disease caused by mutations of putative acyltransferase genes that manifests itself as cardiomyopathy and skeletal myopathy (31). Thus, studies of LCLAT are needed to clarify the regulatory role of CL remodeling in cardiac function.

PG is a precursor for the synthesis of CL and a potential activator of the protein kinase C family, including nuclear protein kinase Cβ_{II} (32). The same group who cloned LCLAT1 found that LPGAT1 catalyzed the synthesis of PG from lyso-PG and acyl-CoA (33). The enzyme also possesses the LPLAT motifs. Human LPGAT1 overexpressed in COS-7 cells showed a clear preference for 16:0-, 18:0-, and 18:1-CoAs as donors, which is consistent with the composition of endogenous PG in several tissues. Human LPGAT1 is widely distributed in tissues. The enzyme has the KK*XX* motif and is localized to the ER (33). These two studies are a pioneering achievement in initiating studies on acyltransferases at the molecular level.

LPCAT1 and LPCAT2—We (34) and Chen *et al.* (35) independently discovered LPCAT1 (also called AGPAT9 or AT-like 2), which has LPLAT motifs and catalyzes dipalmitoyl-PC syn-

thesis. The enzyme is expressed mainly in the lung, especially in alveolar type II cells, and its mRNA is increased during the perinatal period. Dipalmitoyl-PC is a major component of pulmonary surfactant, which decreases surface tension, thereby preventing alveolar collapse, small airway closure, and alveolar flooding. Pulmonary surfactant deficiency is recognized to be an important contributing factor in the pathogenesis of infant respiratory distress syndrome, acute respiratory distress syndrome, asthma, and bronchiolitis (36). Thus, LPCAT1 may synthesize the PC of pulmonary surfactant and play a critical role in respiratory physiology. It is of note that LPCAT1 utilizes 18:2- or 18:3-acyl-CoA and exhibits LPGAT activity, reflecting the exact lipid composition of pulmonary surfactant. Further studies are needed to elucidate the exact roles of LPCAT1 *in vivo* and to determine whether there is a direct relationship between LPCAT1 and surfactant lipid synthesis.

LPCAT2 (called LysoPAFAT/LPCAT2 and AT-like 1) was identified by our group as the long-sought lyso-PAF acetyltransferase involved in PAF biosynthesis in the remodeling pathway (37). LPCAT2 also has the LPLAT, EF hand-like, and KK*XX* motifs, which is consistent with its subcellular localization to the ER. The highest level of LPCAT2 expression was observed in resident macrophages and casein-induced neutrophils, followed by skin, colon, spleen, and thioglycolate-induced macrophages. Moreover, in mouse thioglycolate-induced macrophages, the mRNA level of LPCAT2 was increased by treatment with TLR agonists, lipopolysaccharide (a TLR4 agonist) and ODN1826 (a TLR9 agonist), both of which are bacterial cell components. The induction was suppressed by dexamethasone treatment. Because the virus component poly(I:C) (a TLR3 agonist) did not induce LPCAT2 expression, LPCAT2 is induced by bacterial infection but not by viral infection. Surprisingly, LPCAT2 also possesses LPCAT activity using 20:4-CoA as the best donor. The reaction product is 1-*O*-alkyl-2-arachidonoyl-PC, which is a major membrane constituent of inflammatory cells as well as a precursor of lyso-PAF. Lyso-PAF is produced from 1-*O*-alkyl-2-arachidonoyl-PC by PLA$_2$s and used to form PAF (Fig. 1) (3, 38). Thus, LPCAT2 is a critically important enzyme not only in the biosynthesis of PAF (lyso-PAF acetyltransferase activity) but also in membrane homeostasis of inflammatory cells (LPCAT activity). Upon acute inflammatory stimulation with lipopolysaccharide, the activated enzyme utilizes acetyl-CoA more efficiently and produces PAF. It is speculated that LPCAT2 is phosphorylated and activated by p38 mitogen-activated protein kinase (MAPK) dependently because an inhibitor of this kinase abolished the activation of LPCAT2 and endogenous lyso-PAF acetyltransferase (37, 39). Therefore, specific inhibitors of LPCAT2 may be better anti-inflammatory drugs than PAF receptor antagonists because they also inhibit proliferation of inflammatory cells by disturbing membrane biogenesis. It will be important to characterize both acetyltransferase and acyltransferase activities of LPCAT2, including identification of binding sites for each substrate (acetyl-CoA and arachidonoyl-CoA). Additionally, it will be necessary to study the differential regulation of each enzyme activity.

A recent study demonstrated that LPCAT1 catalyzes not only dipalmitoyl-PC synthesis (LPCAT) but also PAF synthesis (lyso-PAF acetyltransferase) (Fig. 1) (40). In mouse macro-

phages, LPCAT1 was neither activated nor up-regulated by lipopolysaccharide stimulation, which was in contrast to LPCAT2. Moreover, both LPCAT1 and LPCAT2 have EF hand-like motifs; however, only LPCAT2 showed Ca^{2+}-dependent activity. Site-directed mutagenesis of LPCAT1 demonstrated that the LPLAT motif 2 is an acyl-CoA-binding site in LPCAT1. Thus, two distinct lyso-PAF acetyltransferases are present: LPCAT1, a constitutively expressed enzyme, and LPCAT2, an inducible enzyme. This relationship is similar to cyclooxygenases 1 and 2, which are constitutively expressed and inducible enzymes, respectively (41). This finding indicates that there are two kinds of PAF remodeling pathways: the inflammatory/inducible (LPCAT2) and non-inflammatory/constitutive (LPCAT1) remodeling pathways (40).

Recently, AGPAT7, an enzyme that is similar to LPCAT1 and LPCAT2, was characterized and renamed as LPEAT2 (also called AGPAT7, LPAATη, and AT-like 3) (42). LPEAT2 possesses LPEAT, LPGAT, LPSAT, and LPCAT activities with 18:1- or 20:4-CoA; however, its small interfering RNA decreased only LPEAT activity in HEK293T cells. Because the enzyme is expressed mainly in the brain, it was suggested that LPEAT2 is an important enzyme for the biogenesis of brain PE. In addition, it has been reported that AGPAT7 (LPEAT2) has LPCAT activity in red blood cells, although its activity is relatively weak (43).

MBOAT Family—MBOAT family members are putative acyltransferases (44, 45). A His residue positioned within a long hydrophobic region of these enzymes is invariant, likely making it one of the active-site residues. DGAT1 (DAG acyltransferase 1) and ACAT1 and ACAT2 (acyl-CoA:cholesterol acyltransferases 1 and 2) are members of the MBOAT family. Interestingly, the family also includes protein acyltransferases, such as Porcupine, Hedgehog acyltransferase, and GUP1 (46–48). Porcupine is required for Ser^{209}-dependent acylation (16:1) of Wnt-3a protein (already palmitoylated at Cys^{77}) for secretion (46). Hedgehog acyltransferase palmitates a Cys residue in Sonic Hedgehog, and this modification is essential for its activity as well as for the generation of a protein gradient in the developing embryo (47). In yeast, the *GUP1* gene is proposed to catalyze the remodeling of the glycosylphosphatidylinositol anchor (48). Most members of the MBOAT family have not been characterized, such as MBOAT3–7 and LRC4 (leukocyte receptor cluster member 4). In the latter half of 2007, five groups independently reported an MBOAT homolog in yeast that showed several LPLAT activities (49–53). Soon thereafter, mammalian MBOAT members were reported in 2008 (45, 54–56).

We (54) and Zhao *et al.* (55) independently discovered LPCAT3 (called MBOAT5), which has LPCAT, LPEAT, and LPSAT activities. Moreover, we showed that LPCAT4 (originally called MBOAT2) possesses LPCAT and LPEAT activities, whereas LPEAT1 (called MBOAT1) exhibits LPEAT and LPSAT activities (54). Thus, these results demonstrate that the MBOAT family is a novel LPLAT family. Mouse LPCAT3 mRNA was detected ubiquitously, with the highest expression seen in the testis. On the other hand, mouse LPCAT4 mRNA was highly expressed in the epididymis, brain, testis, and ovary, and mouse LPEAT1 mRNA was highly expressed in the stomach, epididymis, and colon. LPCAT3 showed higher activities

with polyunsaturated fatty acyl-CoAs 20:4-CoA and 18:2-CoA than with saturated fatty acyl-CoA, whereas LPCAT4 and LPEAT1 had a clear preference for 18:1-CoA (Table 1).

LPCAT3 and LPCAT4 had higher LPCAT activity with 1-acyllyso-PC than with 1-*O*-alkyllyso-PC or 1-*O*-alkenyl-lyso-PC as an acceptor. No clear differences were observed between 1-acyl-LPEAT and 1-*O*-alkenyl-LPEAT activities of each enzyme. Based on the apparent K_m and V_{max} values, LPCAT3 had higher LPCAT activity than LPEAT and LPSAT activities. Similarly, LPCAT4 had higher LPCAT activity than LPEAT activity. LPEAT1 showed similar activities for lyso-PE and lyso-PS. Using 20:4-CoA as a donor, endogenous LPCAT, LPEAT, and LPSAT activities were found to be decreased in B16 melanoma cells transfected with LPCAT3 small interfering RNA. Thus, LPCAT3 appears to be a key enzyme exhibiting LPCAT, LPEAT, and LPSAT activities in B16 cells (54).

LPCAT3, LPCAT4, and LPEAT1 were localized primarily to the ER when enzymes were overexpressed in Chinese hamster ovary cells, which is consistent with having the ER-localized motif KKXX in the C-terminal region. Overexpression of each enzyme induced the formation of unidentified organelles (karmella-like structures). Transmission electron microscopy revealed that the atypical multilayer membrane components were detected in Chinese hamster ovary cells transfected with LPCAT3 cDNA (54). The nature and origin of these structures remain to be clarified.

LPIAT1 (also called MBOA-7, MBOAT7, and LRC4) was identified as the first LPIAT, which catalyzes the incorporation of arachidonic acid and eicosapentaenoic acid into lyso-PI (56). Site-directed mutagenesis of human LPIAT1 demonstrated that a predicted active-site residue, His^{350}, within a long hydrophobic region is important for LPIAT activity. An LPIAT1 mutant of *Caenorhabditis elegans* showed a "bags of worms" phenotype whereby the embryos hatched within the mother, leaving a cuticle sack that contained multiple wriggling larvae.

Recently, MBOAT4 was identified as a ghrelin *O*-acyltransferase, which esterifies Ser^3 of ghrelin, an appetite-stimulating peptide hormone, with an octanoyl group (57). Acylation is important for the growth hormone-releasing activity of ghrelin. Thus, the MBOAT family is composed of enzymes that incorporate fatty acids into amino acid residues and lysophospholipids. It is unknown which amino acid residues or motifs are important in distinguishing between lysophospholipids and proteins as acceptors.

Conclusion

In the last 4 years, many LPLATs 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. In this review, we propose to rename LPLATs to clarify and standardize the nomenclature (Table 1). The possibility that additional LPLATs, with preferences for different acyl-CoAs, contribute to membrane composition and diversity will be addressed in future studies. The existence of multiple LPLATs is reminiscent of the 20 distinct aminoacyl-tRNA synthetases and acyltransferases that help incorporate amino acids into pre-existing polypeptides (58). The redundant and pleiotropic substrate preferences of LPLATs may explain the diver-

sity in membrane glycerophospholipids, which vary among tissues and can change in response to external stimuli. So far, a great deal of effort has been expended in trying to understand the biochemical characteristics of individual enzymes. Further studies will be needed to elucidate the biological roles of these enzymes *in vivo*. It will also be important to analyze enzyme activities with mixed substrates of acyl-CoAs and to determine the functional coupling of acyl-CoA synthetases and acyltransferases. Nevertheless, recent findings with LPLATs go a long way toward answering the questions posed by Dr. Lands alluded to earlier (8) and may will open the door to greater understanding of the biological significance of membrane diversity and asymmetry.

REFERENCES

1. van Meer, G., Voelker, D. R., and Feigenson, G. W. (2008) *Nat. Rev. Mol. Cell Biol.* **9**, 112–124
2. Ishii, S., and Shimizu, T. (2000) *Prog. Lipid Res.* **39**, 41–82
3. Shimizu, T., Ohto, T., and Kita, Y. (2006) *IUBMB Life* **58**, 328–333
4. Yamashita, A., Sugiura, T., and Waku, K. (1997) *J. Biochem. (Tokyo)* **122**, 1–16
5. Schlame, M., Rua, D., and Greenberg, M. L. (2000) *Prog. Lipid Res.* **39**, 257–288
6. Kornberg, A., and Pricer, W. E., Jr. (1953) *J. Biol. Chem.* **204**, 329–343
7. Kennedy, E. P., and Weiss, S. B. (1956) *J. Biol. Chem.* **222**, 193–214
8. Lands, W. E. (2000) *Biochim. Biophys. Acta* **1483**, 1–14
9. Lands, W. E. (1958) *J. Biol. Chem.* **231**, 883–888
10. Waku, K., and Nakazawa, Y. (1972) *J. Biochem. (Tokyo)* **72**, 495–497
11. Van den Bosch, H., and Vance, D. E. (1997) *Biochim. Biophys. Acta* **1348**, 1–2
12. Coleman, R. A., and Lee, D. P. (2004) *Prog. Lipid Res.* **43**, 134–176
13. Lewin, T. M., Wang, P., and Coleman, R. A. (1999) *Biochemistry* **38**, 5764–5771
14. Yamashita, A., Nakanishi, H., Suzuki, H., Kamata, R., Tanaka, K., Waku, K., and Sugiura, T. (2007) *Biochim. Biophys. Acta* **1771**, 1202–1215
15. Yet, S. F., Lee, S., Hahm, Y. T., and Sul, H. S. (1993) *Biochemistry* **32**, 9486–9491
16. Harada, N., Hara, S., Yoshida, M., Zenitani, T., Mawatari, K., Nakano, M., Takahashi, A., Hosaka, T., Yoshimoto, K., and Nakaya, Y. (2007) *Mol. Cell. Biochem.* **297**, 41–51
17. Cao, J., Li, J. L., Li, D., Tobin, J. F., and Gimeno, R. E. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 19695–19700
18. Chen, Y. Q., Kuo, M. S., Li, S., Bui, H. H., Peake, D. A., Sanders, P. E., Thibodeaux, S. J., Chu, S., Qian, Y. W., Zhao, Y., Bredt, D. S., Moller, D. E., Konrad, R. J., Beigneux, A. P., Young, S. G., and Cao, G. (2008) *J. Biol. Chem.* **283**, 10048–10057
19. Nagle, C. A., Vergnes, L., Dejong, H., Wang, S., Lewin, T. M., Reue, K., and Coleman, R. A. (2008) *J. Lipid Res.* **49**, 823–831
20. Wang, S., Lee, D. P., Gong, N., Schwerbrock, N. M., Mashek, D. G., Gonzalez-Baro, M. R., Stapleton, C., Li, L. O., Lewin, T. M., and Coleman, R. A. (2007) *Arch. Biochem. Biophys.* **465**, 347–358
21. West, J., Tompkins, C. K., Balantac, N., Nudelman, E., Meengs, B., White, T., Bursten, S., Coleman, J., Kumar, A., Singer, J. W., and Leung, D. W. (1997) *DNA Cell Biol.* **16**, 691–701
22. Stamps, A. C., Elmore, M. A., Hill, M. E., Kelly, K., Makda, A. A., and Finnen, M. J. (1997) *Biochem. J.* **326**, 455–461
23. Kume, K., and Shimizu, T. (1997) *Biochem. Biophys. Res. Commun.* **237**, 663–666
24. Eberhardt, C., Gray, P. W., and Tjoelker, L. W. (1997) *J. Biol. Chem.* **272**, 20299–20305
25. Lu, B., Jiang, Y. J., Zhou, Y., Xu, F. Y., Hatch, G. M., and Choy, P. C. (2005) *Biochem. J.* **385**, 469–477
26. Hollenback, D., Bonham, L., Law, L., Rossnagle, E., Romero, L., Carew, H., Tompkins, C. K., Leung, D. W., Singer, J. W., and White, T. (2006) *J. Lipid Res.* **47**, 593–604
27. Agarwal, A. K., Arioglu, E., De Almeida, S., Akkoc, N., Taylor, S. I., Bowcock, A. M., Barnes, R. I., and Garg, A. (2002) *Nat. Genet.* **31**, 21–23
28. Cao, J., Liu, Y., Lockwood, J., Burn, P., and Shi, Y. (2004) *J. Biol. Chem.* **279**, 31727–31734
29. Shikano, S., and Li, M. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 5783–5788
30. Agarwal, A. K., Barnes, R. I., and Garg, A. (2006) *Arch. Biochem. Biophys.* **449**, 64–76
31. Schlame, M., Kelley, R. I., Feigenbaum, A., Towbin, J. A., Heerdt, P. M., Schieble, T., Wanders, R. J., DiMauro, S., and Blanck, T. J. (2003) *J. Am. Coll. Cardiol.* **42**, 1994–1999
32. Murray, N. R., and Fields, A. P. (1998) *J. Biol. Chem.* **273**, 11514–11520
33. Yang, Y., Cao, J., and Shi, Y. (2004) *J. Biol. Chem.* **279**, 55866–55874
34. Nakanishi, H., Shindou, H., Hishikawa, D., Harayama, T., Ogasawara, R., Suwabe, A., Taguchi, R., and Shimizu, T. (2006) *J. Biol. Chem.* **281**, 20140–20147
35. Chen, X., Hyatt, B. A., Mucenski, M. L., Mason, R. J., and Shannon, J. M. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 11724–11729
36. Stevens, T. P., and Sinkin, R. A. (2007) *Chest* **131**, 1577–1582
37. Shindou, H., Hishikawa, D., Nakanishi, H., Harayama, T., Ishii, S., Taguchi, R., and Shimizu, T. (2007) *J. Biol. Chem.* **282**, 6532–6539
38. Shindou, H., Ishii, S., Uozumi, N., and Shimizu, T. (2000) *Biochem. Biophys. Res. Commun.* **271**, 812–817
39. Shindou, H., Ishii, S., Yamamoto, M., Takeda, K., Akira, S., and Shimizu, T. (2005) *J. Immunol.* **175**, 1177–1183
40. Harayama, T., Shindou, H., Ogasawara, R., Suwabe, A., and Shimizu, T. (2008) *J. Biol. Chem.* **283**, 11097–11106
41. Smith, W. L., and Langenbach, R. (2001) *J. Clin. Investig.* **107**, 1491–1495
42. Cao, J., Shan, D., Revett, T., Li, D., Wu, L., Liu, W., Tobin, J. F., and Gimeno, R. E. (2008) *J. Biol. Chem.* **283**, 19049–19057
43. Soupene, E., Fyrst, H., and Kuypers, F. A. (2008) *Proc. Natl. Acad. Sci. U. S. A.* **105**, 88–93
44. Hofmann, K. (2000) *Trends Biochem. Sci.* **25**, 111–112
45. Shimizu, T. (2009) *Annu. Rev. Pharmacol. Toxicol.*, in press
46. Takada, R., Satomi, Y., Kurata, T., Ueno, N., Norioka, S., Kondoh, H., Takao, T., and Takada, S. (2006) *Dev. Cell* **11**, 791–801
47. Chen, M. H., Li, Y. J., Kawakami, T., Xu, S. M., and Chuang, P. T. (2004) *Genes Dev.* **18**, 641–659
48. Bosson, R., Jaquenoud, M., and Conzelmann, A. (2006) *Mol. Biol. Cell* **17**, 2636–2645
49. Riekhof, W. R., Wu, J., Jones, J. L., and Voelker, D. R. (2007) *J. Biol. Chem.* **282**, 28344–28352
50. Benghezal, M., Roubaty, C., Veepuri, V., Knudsen, J., and Conzelmann, A. (2007) *J. Biol. Chem.* **282**, 30845–30855
51. Jain, S., Stanford, N., Bhagwat, N., Seiler, B., Costanzo, M., Boone, C., and Oelkers, P. (2007) *J. Biol. Chem.* **282**, 30562–30569
52. Tamaki, H., Shimada, A., Ito, Y., Ohya, M., Takase, J., Miyashita, M., Miyagawa, H., Nozaki, H., Nakayama, R., and Kumagai, H. (2007) *J. Biol. Chem.* **282**, 34288–34298
53. Chen, Q., Kazachkov, M., Zheng, Z., and Zou, J. (2007) *FEBS Lett.* **581**, 5511–5516
54. Hishikawa, D., Shindou, H., Kobayashi, S., Nakanishi, H., Taguchi, R., and Shimizu, T. (2008) *Proc. Natl. Acad. Sci. U. S. A.* **105**, 2830–2835
55. Zhao, Y., Chen, Y. Q., Bonacci, T. M., Bredt, D. S., Li, S., Bensch, W. R., Moller, D. E., Kowala, M., Konrad, R. J., and Cao, G. (2008) *J. Biol. Chem.* **283**, 8258–8265
56. Lee, H. C., Inoue, T., Imae, R., Kono, N., Shirae, S., Matsuda, S., Gengyo-Ando, K., Mitani, S., and Arai, H. (2008) *Mol. Biol. Cell* **19**, 1174–1184
57. Yang, J., Brown, M. S., Liang, G., Grishin, N. V., and Goldstein, J. L. (2008) *Cell* **132**, 387–396
58. Ibba, M., and Soll, D. (2000) *Annu. Rev. Biochem.* **69**, 617–650

Recent progress on Acyl-CoA:lysophospholipid acyltransferase research*

Hideo Shindou[1], Daisuke Hishikawa, Takeshi Harayama, Koichi Yuki, and Takao Shimizu

From the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Running footline: LPLATs in the Lands' cycle

[1]Corresponding author: Hideo Shindou, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; Tel: +81 3 5802 2925; Fax: +81 3 3813 8732; E-mail: hshindou-tky@umin.ac.jp

[2]The abbreviations used are: PAF, platelet-activating factor; G3P, glycerol-3-phosphate; PLA_2, phospholipase A_2; LPLAT, lysophospholipid acyltransferase; LPA, lysophosphatidic acid; GPAT, G3P acyltransferase; PA, phosphatidic acid; LPAAT, lyso-PA acyltransferase, DAG, diacylglycerol; TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; CDP, cytidine diphosphate; PI, phosphatidylinositol; PG, phosphatidylglycerol; CL cardiolipin; AGPAT, 1-acylglycerol-3-phophate O-acyltransferase; LPCAT, lyso-PC acyltransferase, LPIAT, lyso-PI acyltransferase; LCLAT, lyso-CL acyltransferase; LPGAT, lyso-PG acyltransferase; LPEAT, lyso-PE acyltransferase; MBOAT, membrane bound O-acyltransferase; DGAT, DAG acyltransferase; ACAT, cholesterol acyltransferase; LPSAT, lyso-PS acyltransferase.

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 which contribute to membrane asymmetry and diversity.

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

I. 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[2]) 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 (G3P) 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[2]s (PLA[2]s) and lysophospholipid acyltransferases (LPLATs) (Fig. 1) (2, 6, 7, 10). Since 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 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.

II. Acyltransferases in the *de novo* pathway (Kennedy pathway)

In the *de novo* pathway of glycerophospholipid biosynthesis, lysophosphatidic acid (LPA) is first formed from G3P by G3P acyltransferases (GPATs) (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 (DAG), which is subsequently converted to triacylglycerol (TAG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). Some of them are changed into phosphatidylserine (PS). The other glycerol derivative is cytidine diphosphate diacylglycerol (CDP)-DAG, 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). Since 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).

(i) 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 TAG synthesis. The mitochondrial GPATs are regulated nutritionally and hormonally (12). A recent review described the cloning of GPATs (7).

(ii) 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 towards 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).

16

LPCAT1 also has weak LPAAT activity (see section III-ii). A recent review described the cloning of LPAATs (7).

(iii) 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 homologue has not been found (Fig. 2). These gene products have not yet been analyzed in detail.

III. Acyltransferases in the remodeling pathway (Lands' cycle)

Kennedy and Weiss 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$_2$s and LPLATs (Fig. 1) (2, 6, 7). Characterization of PLA$_2$s has been more extensive (2) than that of LPLATs. Recently, however, several LPLATs have been cloned and characterized as remodeling enzymes (7).

(i) 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. 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) (17). 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, and as does the complete specificity of the enzymes. CL 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

17

activator of the protein kinase C family, including the nuclear 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).

(ii) 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. independently discovered LPCAT1 (also called AGPAT9 or AT like 2), which catalyzes disaturated-PC and disaturated-PG synthesis (Fig. 2) (21, 22). 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.