A Novel Cardiolipin-remodeling Pathway Revealed by a Gene Encoding an Endoplasmic Reticulum-associated Acyl-CoA:Lyso cardiolipin A cetyltransferase (ALCAT1) in Mouse*

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Cardiolipin is a major membrane polyglycerophospholipid that is required for the reconstituted activity of a number of key mitochondrial enzymes involved in energy metabolism. Cardiolipin is subjected to remodeling subsequent to its de novo biosynthesis to attain appropriate acyl composition for its biological functions. Yet, the enzyme(s) involved in the remodeling process have not been identified. We report here the identification and characterization of a murine gene that encodes an acyl-CoA:Lyso cardiolipin acetyltransferase 1 (ALCAT1). Expression of the ALCAT1 cDNA in either insect or mammalian cells led to a significant increase in acyl-CoA:Monolysocardiolipin acetyltransferase and acyl-CoA:Di lyso cardiolipin acetyltransferase activities that exhibited a dependence upon ALCAT1 enzyme levels. The recombinant ALCAT1 enzyme recognizes both monolysocardiolipin and di lyso cardiolipin as substrates with a preference for linoleoyl-CoA and oleoyl-CoA as acyl donors. In contrast, no significant increases in acyltransferase activities by the recombinant ALCAT1 were detected against either glycerol-3-phosphate or a variety of other lysophospholipids as substrates, including lyso phosphatidylcholine, lyso phosphatidylethanolamine, and lyso phosphatidylserine. Immunocytohistochemical analysis showed that the ALCAT1 enzyme is localized in the endoplasmic reticulum, which is supported by a significant ALCAT activity in isolated liver and heart microsomes. Northern blot analysis indicates that the mouse ALCAT1 is widely distributed, with the highest expression in heart and liver. In support of a role for ALCAT1 in maintaining heart function, the gene is expressed most dominantly by the linoleoyl group (C18:2) (6). The unique fatty acyl composition is believed to be important for its proper biological functions. The hydrophobic double-unsaturated linoleic diacylglycerol species seems to be required for the high affinity binding of cardiolipin to proteins (7). Thus, alteration in molecular species composition of cardiolipin was shown to affect the activities of cytochrome c oxidase and other electron transport chain enzymes (8, 9). However, the formation of the unique fatty acyl content of cardiolipin does not occur during de novo biosynthesis, because the enzymes of the cardiolipin biosynthetic pathway lack appropriate substrate selectivity (10–12). Hence, newly synthesized cardiolipin is believed to undergo a remodeling process to achieve its appropriate acyl content. Two distinct mechanisms have been proposed to carry out the remodeling process. The first involves de-acylation by phospholipase A2 to lyso cardiolipin followed by reacylation of lysocardiolipin (1). An enzyme responsible for the acylation of monolysocardiolipin to cardiolipin, acyl-CoA:Monolyso cardiolipin acyltransferase (MLCL AT),1 has been characterized in the rat liver and heart, and was recently purified from pig liver mitochondria fraction (13–15). The alternative mechanism proposed for cardiolipin remodeling involves transacylation of acyl groups from phosphatidylcholine or phosphatidylethanolamine using both cardiolipin and monolysocardiolipin as substrates (16). Thus far, cumulative information of the remodeling process is not conclusive due to the lack of a cloned gene that encodes the enzyme(s).

In the present study, we report the identification and characterization of a murine gene encoding an acyl-CoA:Lyso cardiolipin acetyltransferase 1 (ALCAT1) that possesses both MLCL AT and acyl-CoA:Dilysocardiolipin acetyltransferase (DLCL AT) activities. The recombinant ALCAT1 expressed in both Spodoptera frugiperda 9 (SF9) insect cells and mammalian cells demonstrated predominant activity and selectivity toward linoleoyl-CoA and oleoyl-CoA. Consistent with a major role of cardiolipin in heart function, the gene is expressed most abundantly in heart and liver. In comparison to the two known

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1 The abbreviations used are: MLCL AT, acyl-CoA:Monolysocardiolipin acyltransferase; ALCAT1, acyl-CoA:Lyso cardiolipin acyltransferase 1; DLCL AT, acyl-CoA:Dilysocardiolipin acyltransferase; SF9, Spodoptera frugiperda 9; ER, endoplasmic reticulum; TLC, thin layer chromatography; GPAT, glycerol-3-phosphate acyltransferase; MLCL, monolysocardiolipin; PA, phosphatidic acid; PI, phosphatidylinositol.
mechanisms involved in cardiolipin remodeling in the mitochondria, subcellular localization studies indicate that ALCAT1 is localized in the endoplasmic reticulum (ER), thus revealing a novel role of ER in cardiolipin remodeling.

**EXPERIMENTAL PROCEDURES**

**Cloning ofFull-length Lysocardiolipin Acyltransferase cDNA**—The full-length mouse ALCAT1 cDNA was cloned by PCR amplification using forward primer 5′-CTGCTCCTTGGCAGCTCCGCTC-3′ and for reverse primer 5′-CAAAAGACTGACATTCCCCGTGACA-3′, designed from two mouse expressed sequence tag (EST) sequences (GenBankTM accession numbers BY718809 and B1558124) and Marathon-Ready cDNA prepared from 7-day mouse embryo (BD Biosciences Clontech, Palo Alto, CA). Amplification was performed by PCR for 35 × 30 s at 94 °C for 30 s, 62 °C for 30 s, and 72 °C 2 min 10 s, resulting in a 1.3-kb cDNA product. The PCR product was cloned into the pPCR-script Amp SK(+) vector (Strategene) and sequenced. The human ALCAT1 cDNA inserts were subcloned from the fusion gene into the NotI and EcoRV sites of pcDNA3.1(+)/Hygro mammalian expression vector (Invitrogen). Transient expression of ALCAT1 in COS-7 cells was carried out with Fugene 6 (Roche Diagnostics). Expression of FLAG-tagged ALCAT1 (in 20 μg of membrane protein) was measured by Western blot analysis using an anti-FLAG antibody. Subcellular localization of FLAG-tagged ALCAT1 in COS-7 cells was carried by immunocytostaining chemistry using a protocol as described previously (17).

**In Vitro Acyltransferase Assays**—GPAT activity was determined by measuring the conversion of glycerol 3-phosphate to radiolabeled 1-acyl-sn-glycerol-3-phosphate in the presence of [14C]palmitoyl-CoA as described previously (18, 19). MLCL AT, DLCL AT, or other lysophospholipid acyltransferase was determined by measuring the incorporation of radiolabeled acyl moieties of acyl-CoAs (acyl donors) into phospholipids in the presence of MLCL, DLCL, lysophosphatidic acid (LPA), lysophosphatidylycholine (LPC), lysophosphatidylethanolamine (LPE), and lysophosphatidylserine (LPS) (14, 15). The reaction mixture contained 50 mM Tris/HCl, pH 7.0, 200 μM lysophospholipids, 25 μM [14C]acyl-CoA (50 mCi/mmol, American Radiolabeled Chemicals, Inc), and enzyme preparation (100–200 μg) in a total volume of 200 μl. The reaction was incubated at room temperature for 15–30 min. The lipids were extracted using a method as described previously (14, 15). The extract was separated by thin layer chromatography (TLC) with chloroform:hexane:methanol:acetic acid (50:30:10:5, v/v/v/v) or chloroform:methanol:water (65:25:4, v/v/v) or chloroform:methanol:water (65:25:4, v/v/v). In some experiments, separation of cardiolipin from monolysocardiolipin from other lipids was confirmed by two-dimensional TLC analysis as described (16). After separation, TLC plates were exposed to a PhosphorImager screen to visualize the radiolabeled products with a Molecular Dynamics STORM 860 Scanner (Sunnyvale, CA). The enzymatic products were identified and quantified using authentic standards by exposure to L1 vapor. Quantification of cardiolipin remodeling was carried out by scraping into scintillation vials followed by scintillation counting with a Beckman LS 6500 Scintillation System (Fullerton, CA). The specific activity of the enzyme was calculated on the basis of pmol or nmol of lipids product formed per minute of incubation time per mg protein.

**Fig. 1. Features of the predicted ALCAT1 peptide sequence.** A, the predicted peptide sequence of the mouse ALCAT1 enzyme. A signal peptide located at the N terminus is underlined. The consensus sequences for glycerophospholipid acyltransferases are highlighted by a red font, and a predicted N-glycosylation site is highlighted by a bold font. Sequence motif (KKXX) predicted for ER localization is located at the extreme C terminus. B, hydrophobicity plot of the mouse ALCAT1 peptide. A predicted transmembrane region (TM) at the C-terminal portion of the protein is indicated with a bar.

In mammalian cells, ALCAT1 is localized in the endoplasmic reticulum (ER), thus revealing a novel role of ER in cardiolipin remodeling.
of protein (pmol or nmol/mg protein/min). All quantitative data were expressed as mean ± S.E. Statistical analyses for differences between two groups were carried out using a Student’s t test.

**RESULTS**

Identification and Cloning of the Mouse ALCAT1 Gene—The mouse ALCAT1 gene was identified based upon sequence homology with a predicted rat acyltransferase gene (GenBank™ accession no. XM_233853) that carries the consensus sequence for glycerol-3-phosphate acyltransferase (GPAT) (20). A 1.3-kb cDNA clone encoding the full-length mouse ALCAT1 enzyme was cloned by PCR amplification using primers designed from two mouse EST sequences (BY718809 and BI558124) that match the rat gene and a cDNA library from 7-day-old mouse embryo as template. The open reading frame of the mouse ALCAT1 gene predicts a 376-amino acid protein of 44.4 kDa that carries features for a transmembrane protein and motifs conserved among members of the GPAT family (20). In contrast to an 8-fold increase of GPAT activity observed from the recombinant GPAT1 expressed in Sf9 cells, no significant increase in GPAT activity was detected from recombinant ALCAT1 protein expressed in Sf9 cells relative to that from Sf9 cells infected with the wild-type baculovirus as negative control (Fig. 3A). Because the enzyme is most abundantly expressed in the heart, we next investigated whether the ALCAT1 enzyme possesses MLCL AT activity using oleoyl-CoA and monososcardiolipin (MLCL) as substrates. As a result, a 9-fold increase in MLCL AT activity was observed from recombinant ALCAT1 enzyme expressed in Sf-9 cells relative to that from cells infected with the wild-type baculovirus or recombinant baculovirus expressing the GPAT1 cDNA, as assessed by a representative TLC analysis (Fig. 3B, lane 7 versus lanes 5 and 6). When expressed as absolute units (pmol/mg protein/min), the MLCL AT activity of ALCAT1 expressed in Sf-9 cells (237 ± 20, n = 4) was also significantly higher than that from Sf-9 cells infected with wild-type baculovirus (42 ± 4, n = 4) or recombinant baculovirus that expresses GPAT1 (33 ± 4, n = 4) (Fig. 3D, left, p < 0.01). The MLCL AT activity of the ALCAT1 enzyme is MLCL-dependent, because no labeled cardiolipin was detected in the absence of exogenous MLCL (Fig.

**Functional Analysis of ALCAT1 Expressed in Insect Cells**—Recombinant ALCAT1 protein was expressed in Sf9 insect cells for functional characterization of the enzyme. A GPAT enzymatic assay was initially undertaken to determine whether the polypeptide encoded by ALCAT1 cDNA possesses GPAT activity, because the predicted ALCAT1 protein carries motifs that match all four conserved domains among members of the GPAT family (20). In contrast to an 8-fold increase of GPAT activity observed from the recombinant GPAT1 expressed in Sf9 cells, no significant increase in GPAT activity was detected from recombinant ALCAT1 protein expressed in Sf9 cells relative to that from Sf9 cells infected with the wild-type baculoviruses as negative control (Fig. 3A). Because the enzyme is most abundantly expressed in the heart, we next investigated whether the ALCAT1 enzyme possesses MLCL AT activity using oleoyl-CoA and monososcardiolipin (MLCL) as substrates. As a result, a 9-fold increase in MLCL AT activity was observed from recombinant ALCAT1 enzyme expressed in Sf-9 cells relative to that from cells infected with the wild-type baculovirus or recombinant baculovirus expressing the GPAT1 cDNA, as assessed by a representative TLC analysis (Fig. 3B, lane 7 versus lanes 5 and 6). When expressed as absolute units (pmol/mg protein/min), the MLCL AT activity of ALCAT1 expressed in Sf-9 cells (237 ± 20, n = 4) was also significantly higher than that from Sf-9 cells infected with wild-type baculovirus (42 ± 4, n = 4) or recombinant baculovirus that expresses GPAT1 (33 ± 4, n = 4) (Fig. 3D, left, p < 0.01). The MLCL AT activity of the ALCAT1 enzyme is MLCL-dependent, because no labeled cardiolipin was detected in the absence of exogenous MLCL (Fig.

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3B, lane 4 versus lane 7). In addition to MLCL, the mouse
ALCAT1 also recognized DLCL as its substrate, as demon-
strated by a 3.2-fold increase in radiolabeled monolysocardio-
lipin catalyzed by recombinant ALCAT1 expressed in Sf9 cells
as compared with the controls (Fig. 3C, lane 3 versus
lanes 1 and 2). When expressed in absolute units (pmol
MLCL/mg of protein/min), the DLCL AT activity (Fig. 3D,
right) of ALCAT1 expressed in Sf9 cells (550 ± 26, n = 4)
was more than 3-fold higher than the controls (138 ± 22 and 128 ± 21, respectively; n = 4, p < 0.01). The enzyme
products were authenticated by a two-dimensional TLC
analysis, and the results confirmed that cardiolipin (CL)
and MLCL were the only products migrating at the
positions of CL and MLCL on one-dimensional TLC,
respectively (Fig. 3E). In contrast, no significant increases
in acyltransferase activities were detected against a variety
of other lysophospholipids as acyl receptors, including LPC,
LPE, and LPS (Fig. 3F), although a slight increase in radiolabeled
phosphatidic acid (PA) was observed in the presence of LPA
and [14C]oleoyl-CoA (Fig. 3F) when compared with the controls.

The recombinant ALCAT1 activity was heat-labile (Fig. 3G,
lane 4 versus lane 2) and demonstrated a CoA-dependence (Fig.
3G), because no radiolabeled cardiolipin was detected when
[14C]oleoyl-CoA was replaced by [14C]oleic acid as substrate
(Fig. 3G, last two lanes).

Analysis of the Preference of ALCAT1 toward Different Fatty
Acyl-CoAs—A variety of acyl-CoA derivatives, including n-oct-
tanoyl-CoA (C8:0), lauroyl-CoA (C12:0), palmitoyl-CoA (C16:0),
stearyl-CoA (C18:0), oleoyl-CoA (18:1), linoleoyl-CoA (18:2),
and arachidonyl-CoA (C20:4) were used to determine the pref-
erence of ALCAT1 activities toward different fatty acyl-CoAs
using either MLCL (Fig. 4A) or DLCL (Fig. 4B) as acyl acceptor.
The data were derived from three independent experimen-
tations and were expressed as mean ± S.E. As shown in Fig. 4,
although homogenate from ALCAT1-expressing Sf9 cells cata-
lyzed reacylation of lysocardiolipin with saturated acyl-CoAs of
different chain lengths (C16:0 and C18:0), a significant portion
of the activity was contributed by endogenous enzymes present
in the Sf9 cells, as evidenced by a similar level of ALCAT

Fig. 3. Enzymatic analysis of ALCAT1 expressed in Sf9 cells. Sf9 cells were infected with wild-type baculovirus (Control) or recombinant baculo-
virus that expresses either GPAT1 or ALCAT1 enzyme. A, analysis of GPAT activity of the recombinant ALCAT1 relative to the human
GPAT1 enzyme expressed in Sf9 cells. The data are expressed as mean ± S.E. from three independent experiments. B, TLC analysis for
monolysocardiolipin acyltransferase (MLCL) activity of the recombinant ALCAT1-expressed Sf9 cells in the presence and absence of MLCL
substrate. The embedded numbers represent the relative levels of cardiolipin (CL) or monolysocardiolipin (MLCL). C, TLC analysis for dllyso-
cardiolipin acyltransferase (DLCL) activity of the recombinant ALCAT1 expressed in Sf9 cells in the presence and absence of DLCL. D, quantitative
analysis of MLCL AT and DLCL AT activities of recombinant ALCAT1 expressed in Sf9 cells relative to the negative controls. The values are
mean ± S.E. from four independent experiments. *, p < 0.01, ALCAT1 versus control or GPAT1. E, authentication of MLCL and CL, the enzymatic
products catalyzed by the recombinant ALCAT1, by two-dimensional TLC analysis. The results confirmed that no other radiolabeled phospholipid
moieties co-migrated with MLCL and CL on one-dimensional TLC. F, profiling of substrate specificity by TLC analysis of the recombinant ALCAT1
toward different lysophospholipids, including lyso phosphatidic acid (LPA), lyso phosphatidylcholine (LPC), lyso phosphatidylethanolamine (LPE),
and lyso phosphatidylserine (LPS), *, the migrating position of the authentic standards for each of the phospholipids. G, the recombinant ALCAT1
activity in Sf9 cells was heat-labile and CoA-dependent. FFA, free fatty acid.
ALCAT1 enzyme for the two unsaturated C18 acyl donors expressing baculovirus relative to that from the wild-type activity was observed from Sf9 cells infected with the ALCAT1—when either oleoyl-CoA or linoleoyl-CoA was used as acyl donor, a 5- to 10-fold increase in ALCAT activities (DLCL AT) using oleoyl-CoA as acyl donor at 20:4 linoleoyl-CoA; and 20:4, arachidonoyl-CoA.

Expression of ALCAT1 in Mammalian Cells—To investigate the expression and activity of recombinant ALCAT1 in mammalian cells, and to correlate ALCAT1 activity with the level of expressed ALCAT1 protein, a FLAG-tagged version of ALCAT1 cDNA was expressed in COS-7 cells to measure the level of ALCAT1 protein (Western blot using anti-FLAG antibody) and ALCAT activities (DLCL AT) using oleoyl-CoA as acyl donor at different time points after transfection. The FLAG-tagged version of ALCAT1 migrated on SDS-PAGE with an apparent molecular mass of ~44 kDa (Fig. 5A), which is consistent with the molecular mass of 44.4 kDa predicted from the open reading frame of the mouse ALCAT1 gene. The levels of ALCAT1 protein expression reached a peak at 48 h (Fig. 5A), which correlated well with the profile of ALCAT1 activities (Fig. 5B), further confirming the specificity of the enzyme.

Subcellular Localization of ALCAT1—To define the subcellular localization of ALCAT1, we next performed an immunocytohistology study of the FLAG-tagged ALCAT1 in COS-7 cells. Forty-eight hours after transfection, cells were processed for mitochondrial staining with MitoTracker Red CMXRos or indirect immunofluorescence staining with antibodies specific for the FLAG peptide and calnexin, a resident ER transmembrane protein serving as an ER marker (21). Cells were also counterstained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize nuclei (blue). FLAG-ALCAT1 expressed in COS-7 cells displayed a perinuclear and punctated pattern (Fig. 6, A and D) that did not colocalize with MitoTracker Red CMXRos-stained mitochondria (Fig. 6B), as evidenced in Fig. 6F by the well separated green (FLAG-ALCAT1) and red (mitochondria) colors in the merged image. In contrast, FLAG-tagged ALCAT1 protein co-localized well with the ER marker, calnexin (Fig. 6E), as evidenced in Fig. 6F by the yellow color in the merged image. The results are consistent with the KXXX motif, an ER retention signal, at the C-terminal end of the ALCAT1 peptide predictive for ER localization.

Comparative Analyses of ALCAT Activities Associated with Isolated Microsomes and Mitochondria Fractions from the Mouse Liver and Heart—The finding that ALCAT1 was localized to ER encouraged us to examine further the mitochondrial and microsomal ALCAT activities in mouse tissues. The subcellular fractions were prepared as described (24). By using oleoyl-CoA or linoleoyl-CoA as acyl donors, the MLCL AT and DLCL AT activities in mouse liver and heart mitochondria and microsomes were determined, respectively. As shown in Fig. 7 by a representative TLC image (left side of each panel) and averages from three different preparations of subcellular fractions (right side, bar graph), the isolated microsomes from both liver and heart demonstrated high levels of ALCAT activities, as evidenced by efficient incorporation of the radiolabeled oleoyl or linoleoyl moiety into cardiolipin (Fig. 7A) and monolysocardiolipin (Fig. 7B), respectively. Similarly, ALCAT activities were also detected from the isolated mitochondria from rat liver and heart, but at significantly lower levels. The ALCAT activity of the liver mitochondria was less than 50% of that from the microsomes with either oleoyl-CoA or linoleoyl-CoA as acyl donor, whereas heart mitochondrial activity was ~30–40%
of that from the heart microsomes (Fig. 7, A and B), suggesting a more active role of ER in cardiolipin remodeling. Furthermore, the ALCAT activities associated with liver microsomes demonstrated a different substrate specificity profile from that of mitochondria, as assessed by DLCL AT activity (Fig. 7C). The ALCAT activities associated with mitochondria displayed the highest activity toward stearoyl-CoA (C18:0) (Fig. 7C, left), whereas the ALCAT activities associated with the microsomal fraction preferred oleoyl- and palmitoyl-CoAs as acyl donors (Fig. 7C, right).

The relative purity of the enriched microsomal and mitochondria fractions used in the ALCAT activity analysis was verified by Western analysis using protein markers specific for mitochondria (prohibitin) and microsome (calnexin). When compared with that from the microsome fraction, very low levels (6.6%) of calnexin were detected in the mitochondria fractions from both liver and heart (Fig. 7D, top blot, lanes 1 and 3 versus lanes 2 and 4, respectively). The levels of prohibitin in microsome fractions were only 20–30% of that detected in mitochondria fractions (Fig. 7D, bottom blot, lanes 2 and 4 versus lanes 1 and 3, respectively). Therefore, the microsomal ALCAT activities caused by the cross-contamination of mitochondria are very limited.

**DISCUSSION**

It is well documented that fatty acid remodeling of phospholipids occurs after *de novo* synthesis in mammalian tissues to achieve appropriate fatty acid composition (22, 23). Although an ALCAT enzyme activity was reported from rat liver microsomes more than three decades ago (24), a gene encoding the enzyme has not been identified. In this study, we identified a gene encoding an enzyme that possesses both MLCL AT and DLCL AT activities, designated here as ALCAT1. Evidence that the ALCAT1 gene encodes a lysocardiolipin acyltransferase is provided by the following findings. First, expression of ALCAT1 cDNA in Sf9 cells resulted in 5- to 10-fold increases in radiolabeled cardioliopin product, which depended on the presence of acyl-CoA and monolysocardiolipin or dilysocardiolipin. Absence of the acyl donors or substitution of acyl-CoA with free fatty acid resulted in loss of the activities. Second, the ALCAT activity demonstrated a dose dependence upon levels of ALCAT1 protein expression. Third, the acyltransferase activity displayed a specificity for lysocardiolipin, because no significant increases in acyltransferase activities were observed when tested against either glycerol 3-phosphate or a variety of lysophospholipids including LPC, LPE, and LPS. Finally, the recombinant ALCAT1 demonstrated a preference to linoleoyl-CoA and oleoyl-CoA as acyl donors, which is consistent with the unique fatty acid pattern of C16, found in mammalian cardiolipin (6, 25).

Previously, two enzymatic mechanisms have been proposed to explain the cardiolipin-remodeling process in mitochondria. The first mechanism proposes that cardiolipin remodeling begins with hydrolysis by phospholipase A2 or other as yet unidentified phospholipases that act upon cardiolipin and is completed with reacylation of cardiolipin by an acyl-CoA-dependent acyltransferase. An enzyme activity responsible for the acylation of lysocardiolipin to cardiolipin, either MLCL AT or DLCL AT, has been characterized in the rat liver and heart (14, 24). Although a 74-kDa protein with MLCL AT activity was recently purified with more than 1000-fold enrichment from pig liver mitochondrial fraction (15), the purified MLCL AT failed to demonstrate the much-anticipated specificity for linoleoyl-CoA (15).

The second mechanism proposed for a cardiolipin-remodeling process involves transacylation of acyl groups from phospholipids such as phosphatidylcholine or phosphatidylethanolamine to cardiolipin (13, 16). In contrast to an acyltransferase reaction that catalyzes acylation of cardiolipin, the transacylation reaction uses both cardiolipin and lysocardiolipin as substrates, and displays a clear preference for the linoleyl group as an acyl donor. However, the transacylation reaction demonstrates a much lower efficiency than that from the acyl-CoA-dependent acyltransferase reaction (1 pmol/min/mg versus 200 pmol/min/mg) (13, 16). In addition, the phospholipid-dependent transacylation enzyme(s) does not recognize dilsylocardiolipin as substrate, which is a major product from cardiolipin hydrolysis (16). Intriguingly, although both remodeling processes are shown to take place in isolated mitochondria, only trace amounts of lysocardiolipin were identified in the organelle (26, 27). Furthermore, the MLCL AT activity in heart mitochondria is the lowest among all the tissues examined (14), despite an important role for cardiolipin remodeling in maintaining heart function (28).

In contrast to the two proposed mitochondrial mechanisms involved in cardiolipin remodeling, the murine ALCAT1 enzyme reported in this study is an ER-associated protein, as evidenced by immunocytohistochemical analysis, thus representing a novel cardiolipin-remodeling pathway. Consistent with a role for the ALCAT1 enzyme in cardiolipin remodeling in ER, ALCAT activity was detected toward both MLCL and DLCL in microsomes isolated from liver and heart. It is unlikely that the microsomal ALCAT activity was caused by mitochondrial contamination, because significantly higher ALCAT activity was detected in the microsomes compared with that in mitochondria. This is also confirmed by Western blot analysis using antibodies that recognize protein markers specific for each organelle, showing that cross-contamination is minimal. Our data on the ALCAT1 enzyme as well as on ALCAT activity associated with isolated microsomes are further supported by a previous report (24), which demonstrated significantly higher ALCAT activities in isolated liver microsomes than those in mitochondria. Consistent with our current findings, the previously reported ALCAT activities associated with the isolated liver microsomes also lacked exclusivity to linoleoyl-CoA as the acyl donor and recognized both MLCL and DLCL as substrates (24).
The physiological significance of the cloned ALCAT1 enzyme involved in cardiolipin remodeling in the ER remains elusive, because the organelle only contains ∼1% cardiolipin (29). Cardiolipin is localized predominantly, if not exclusively, in mitochondria that contain all of the enzymes necessary for cardiolipin biosynthesis. However, it cannot be ruled out that ER, the major subcellular organelle for lipid biosynthesis, also contributes to the cardiolipin synthesis and remodeling in vivo. In support of this scenario is the finding that phosphatidylglycerol, an important and direct precursor for cardiolipin synthesis, is synthesized in both ER and mitochondria (30–32). In mammals, ER serves as a major intracellular source of synthesis for most phospholipids, including phosphatidylcholine, phosphatidylserine, sphingomyelin, and phosphatidylethanolamine (5, 33–35), and these phospholipids must be translocated to the mitochondria for their functioning (36, 37). Although little is known about cardiolipin trafficking thus far, exogenous phosphatidylglycerol was reported to be used directly for cardiolipin synthesis (38). Thus, the identification and molecular cloning of enzymes involved in cardiolipin biosynthesis in mammals will help in answering these questions.

Cardiolipin is a predominant glycerophospholipid found in the mitochondria of heart (1, 29), and cardiolipin remodeling is believed to play an important role in maintaining normal heart function (39). Hence, decreased cardiolipin levels are associated with heart diseases caused by hyperthyroidism and aging, and development of anticardiolipin antibody is associated with the onset of thrombocytopenia and recurrent thrombosis (1). Additionally, defective cardiolipin remodeling is part of the pathophysiological events associated with Barth syndrome, a familial disease caused by mutations of a gene encoding a putative phospholipid acyltransferase, and is manifested by cardiomyopathy, skeletal myopathy, and other abnormalities (40, 41). In support of a role for ALCAT1 in maintaining heart function, the ALCAT1 gene is most abundantly expressed in heart. In addition, a unique mRNA splice variant is only present in the heart, although its identity and functional significance remains to be investigated in future studies. More interestingly, the gene is conserved among different mammalian species from fish to humans, but is absent in non-atrium species, such as C. elegans. Thus, the cloning and characterization of the mouse ALCAT1 gene has laid a foundation for future studies on the regulatory role of cardiolipin remodeling in maintaining heart function.
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