**Dysregulation of Plasmalogen Homeostasis Impairs Cholesterol Biosynthesis**

Received for publication, April 6, 2015, and in revised form, September 16, 2015 Published, JBC Papers in Press, October 13, 2015, DOI 10.1074/jbc.M115.656983

Masanori Honsho, Yuichi Abe, and Yukio Fujiki

**From the Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan**

Plasmalogen biosynthesis is regulated by modulating fatty acyl-CoA reductase 1 stability in a manner dependent on cellular plasmalogen level. However, physiological significance of the regulation of plasmalogen biosynthesis remains unknown. Here we show that elevation of the cellular plasmalogen level reduces cholesterol biosynthesis without affecting the isoprenylation of proteins such as Rab and Pex19p. Analysis of intermediate metabolites in cholesterol biosynthesis suggests that the first oxidative step in cholesterol biosynthesis catalyzed by squalene monooxygenase (SQLE), an important regulator downstream of downstream HMG-CoA reductase in cholesterol synthesis, is reduced by degradation of SQLE upon elevation of cellular plasmalogen level. By contrast, the defect of plasmalogen synthesis causes elevation of SQLE expression, resulting in the reduction of 2,3-epoxysqualene required for cholesterol synthesis, hence implying a novel physiological consequence of the regulation of plasmalogen biosynthesis.

Plasmalogen is a subclass of glycerophospholipid harboring vinyl ether-linked alkyl chain at its sn-1 and acyl chain at its sn-2 position. Physiological significance of plasmalogens is highlighted by fatal human diseases defective in plasmalogen synthesis such as Zellweger syndrome and rhizomelic chondrodysplasia punctata (1). Patients with rhizomelic chondrodysplasia punctata manifest clinical features of shortened proximal long bones, rhizomelia, bilateral congenital cataracts, and mental and growth retardation (2), predicting a role of plasmalogens in the development of bone, brain, and eye lens. In addition, studies using plasmalogen-defective mutant Chinese hamster ovary (CHO) cells revealed that plasmalogens are important for the esterification of cholesterol (3). Abnormality of other processes including myelination, paranode organization, and purkinje cell innervation is shown in a knock-out mouse defective in dihydroxyacetone phosphate acyltransferase, an essential enzyme for the synthesis of plasmalogens (4).

Plasmalogen synthesis comprising the seven-step reactions is initiated in peroxisomes. Formation of the ether-bond is catalyzed by the peroxisomal matrix protein, alkyl-dihydroxyacetonephosphate synthase (ADAPS),

*This work was supported in part by a CREST grant (to Y. F.) from the Science and Technology Agency of Japan, grants from the Global COE Program and Grants for Excellent Graduate Schools from The Ministry of Education, Culture, Sports, Science, and Technology of Japan, Grants-in-Aid for Scientific Research 23570236 and 26440102 (to M. H.) and 24247038, 25112518, 25116717, 26116007, and 15K14511 (to Y. F.), Kyushu University Interdisciplinary Programs in Education and Projects in Research Development (to M. H. and Y. F.), the Takeda Science Foundation (to M. H. and Y. F.), and the Japan Foundation for Applied Enzymology (to Y. F.). The authors declare that they have no conflicts of interest with the contents of this article.

2 The abbreviations used are: ADAPS, alkyl-dihydroxyacetonephosphate synthase; DOS, 2,3;22,23-diepoxysqualene; 24,25-EC, 24,25-epoxycholesterol; Etn, ethanoloamine; Far1, fatty acyl-CoA reductase 1; GPE, glycerophosphoethanolamine; HMGCR, HMG-CoA reductase; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; LSS, lanosterol synthase; MARCH6, membrane-associated RING finger 6; MOS, 2,3-diepoxysqualene; PlsEtn, purified plasmalogens; SM, sphingomyelin; SQLE, squalene monooxygenase; SREBP, sterol regulatory element-binding protein.
terol biosynthesis (10–12). In the present study, we investigated if the cellular plasmalogen level plays a role in cholesterol homeostasis by modulating the cellular level of plasmalogens. We report here a novel consequence of cellular plasmalogen level in cholesterol homeostasis.

**Experimental Procedures**

**Materials**—Restriction enzymes and DNA-modifying enzymes were purchased from Nippon Gene and Takara. Ham’s F-12 and DMEM were from Invitrogen. [14C]Acetate and [14C]palmitate were purchased from Moravek Biochemicals. Ethanolamine (EtN), lovastatin, Ro48-8071, 2,3-monoepoxycholesterol (24,25-EC) was purchased from Sigma. 24,25-Epoxycholesterol (24,25-EC) was used for the metabolic labeling with [14C]acetate and [14C]palmitate. F-12 medium supplemented with 10% fetal bovine serum in 5% CO2 and 95% air (13, 14). These culture mediums were used for the enzymatic method using an Amplex Red cholesterol assay software (Multi Gauge, Fuji Film). Plasmalogens and phosphatidylethanolamine were analyzed by TLC (6, 7, 13) or LC-ESI-MS/MS (17). Cellular free cholesterol was determined by an LAS-4000 imaging analyzer and quantified using an image analyzer (3.0 software (Fuji Film).

**Construction of Expression Vector for MARCH6 and SQLE**—First strand cDNA was prepared from total RNA of HeLa cells. Human MARCH6 was amplified using a RT product as a template with a set of primers, HsMARCH6-Fw.NotI (5’-ccgccccgccggccggaggaagacatatgtag-3’) and HsMARCH6-Rv.SalI (5’-ctcggcctgcgctgctgctgctg-3’) and digested with NotI and SalI. The resultant fragment was cloned into the NotI-XhoI site of pcDNA3.1/Zeo/PMP22-Myc6 and termed pcDNA3.1/Zeo/MARCH6-Myc6. pcDNA3.1/Zeo/MARCH6C9A-Myc6 was generated by inverse PCR using a set of primers HsMARCH6.C9A.Fw (5’-ggagggagacatacgctggtggtggggggcag-3’) and HsMARCH6.C9A.Rv (5’-ggagggagacatacgctggtggtggggggcag-3’). Human SQLE was likewise amplified using a set of primers, HsSQLE.Fw.NotI (5’-gggcggcgcagttgagctgggatatggcagc-3’) and HsSQLE.Rv.Spel (5’-ctcagtagatgacatacattctctg-3’), and was digested with NotI and SpeI. The resultant fragment was cloned into the NotI-Nhel site of pUCD2HygSR.PEX16-HA2 (21).

**Immunoprecipitation—SQLE-HA2**—MARCH6-Myc6 and MARCH6C9A-Myc6 were transfected into CHO cells as described (22) and cultured for 2 days in the presence or absence of EtN. The cells were lysed for 5 min on ice with ice-cold phosphate-buffered saline containing 0.2% Triton X-100 and a mixture of protease inhibitors, and further solubilized at 4°C for 20 min. After centrifugation, cell lysates were subjected to immunoprecipitation using rabbit anti-Myc antibody. Polyclonal antibody to myc peptide was raised in rabbits by injection of the c-Myc peptide, CYILSVQAEEQKLISEEDL. siRNA-mediated Knockdown of MARCH6—MARCH6 knockdown in HeLa cells was performed using siRNA purchased from MISSION RNA (Sigma). EGFP siRNA designed by StealthTMsiRNA was used for a control (Invitrogen). Target sequences of siRNA are as follows: human MARCH6, 5’-CTT-AGTCCATCGCGGATT-3’ and EGFP, 5’-CACATGAGACGCCGACTCTTCT-3’.

**Real-time PCR**—Extraction of total RNA from cells, synthesis of first-strand cDNA, and quantitative real-time PCR were performed using the housekeeping gene porphobilinogen deaminase (PBGD) (23) or ribosomal protein 3 (RPL3) (24) as internal control (17). Primers for Chinese hamster SQLE (12) and LSS (25), and human LSS and HMGC (24) were listed in supplement Table S1.

**Data Presentation**—Quantitative data were shown as mean ± S.D. from three independent experiments.

**Results**

**Elevation of Plasmalogen Level Reduces Cholesterol Synthesis**—To explore physiological consequences of the homeostasis of plasmalogens, cellular plasmalogens were elevated by supplementing CHO-K1 cells with purified plasmalogens (PlsEtN) or EtN and assessed the biosynthesis of lipids by labeling with either [14C]acetate or [14C]palmitate. Supplementation of EtN increased cellular plasmalogens and phosphatidylethanolamine about 1.5 times more than those in CHO-K1 cells. When cells were cultured in the presence of

---

3 Y. Yagita and Y. Fujiki, unpublished data.
Plasmalogens Regulate Cholesterol Biosynthesis

PlsEtn, plasmalogens were increased nearly by 2-fold, whereas phosphatidylethanolamine levels were not altered (Fig. 1, A and C). By liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) analysis, plasmalogens containing 18:0 and 18:1, but not 16:0 fatty alcohols at the respective sn-1 position were shown to be increased in the presence of PlsEtn, which was most likely due to the lesser amount of bovine brain-derived plasmalogens containing 16:0 fatty alcohol (data not shown). In contrast, all species of plasmalogens were elevated in the presence of Etn (Fig. 1 D), when CHO-K1 cells were metabolically labeled with [14C]acetate in the presence of Etn or PlsEtn, cholesterol synthesis was significantly reduced, whereas synthesis of glycerophospholipids including phosphatidylethanolamine and serine- and inositol-containing phospholipids (phosphatidylserine and phosphatidylinositol, respectively) were not altered (Fig. 1, A and B). In these conditions, cellular free cholesterol was not altered (Table 1). Similarly, reduction of cholesterol synthesis was also observed in HeLa and HEK293 cells upon culturing with Etn (Fig. 2, A and B). The cellular level of plasmalogens in HeLa and HEK293 cells was increased about 1.5 times as compared with that in the untreated respective cells (Fig. 2C). Taken together, these results strongly suggest that cholesterol biosynthesis is specifically reduced by elevation of the cellular level of plasmalogens.

**Elevation of Plasmalogen Level Down-regulates Cholesterol Synthesis Steps at Post-isoprenoid Biosynthetic Pathway—We further investigate which step of the cholesterol synthesis is affected upon elevation of plasmalogens.**

**TABLE 1**

| Cell line | Treatment | Free cholesterol<sup>b</sup> (μg/mg protein) | Free cholesterol<sup>a</sup> (μg/mg protein) |
|-----------|-----------|---------------------------------------------|---------------------------------------------|
| CHO-K1    | PlsEtn    | 25.4 ± 1.3                                  | 25.4 ± 1.3                                  |
| CHO-K1    | Etn       | 18.2 ± 1.9                                  | 18.2 ± 1.9                                  |
| CHO-K1    | Cholesterol | 32.0 ± 2.4                          | 32.0 ± 2.4                                  |
| ZPEG251   | Cholesterol | 34.3 ± 1.3                          | 34.3 ± 1.3                                  |

<sup>a</sup> Cell lines were cultured with plasmalogens purified from bovine brain, Etn, ethanolamine, and cholesterol as described under "Experimental Procedures."

<sup>b</sup> Free cholesterol in cell lines was determined by the enzymatic method using an Amplex Red cholesterol assay kit as described under "Experimental Procedures."

<sup>c</sup> Values are mean ± S.D.
Plasmalogens Regulate Cholesterol Biosynthesis

Figure 2. Elevation of plasmalogens causes reduction of cholesterol synthesis in cell lines. A, HeLa and HEK293 cells were cultured for 48 h in the absence (−) or presence (+) of 5 or 2 μM Etn, respectively. Cells were metabolically labeled with [14C]palmitate for the last 5 h during this treatment. Biosynthesis of cholesterol (upper panel) and the level of 2-acyl-GPE (lower panel) are shown. B, synthesis of cholesterol in HeLa and HEK293 cells upon elevation of plasmalogens (open bar) is represented by taking those as 100 in absence (−) or presence (+) of Etn. *; p < 0.05; t test versus a control. C, total amounts of plasmalogens (solid bar) and phosphatidylethanolamine (PE) (open bar) in HeLa (left panel) and HEK293 (right panel) cells cultured in the presence (+) or absence (−) of Etn are shown. In HeLa cells, *; p < 0.01; t test versus a control. In HEK293 cells, *; p < 0.05; t test versus a control.

Figure 3. Elevation of cellular plasmalogens suppresses the post-squalene pathway in cholesterol synthesis. A, CHO-K1 cells were cultured for 48 h with Etn (lanes 2 and 5) or treated with 10 μM lovastatin (Lov) for 5 h (lanes 1 and 4) and prepared cytosolic (Cyt) and organelle (Org) fractions. Farnesylation of Pex19p and geranylgeranylation of Rab4 and Rab5 were verified by immunoblotting with the respective antibodies. Pex3p and lactate dehydrogenase (LDH) were detected by exposing to iodine vapor. Synthesis of squalene is represented by taking as 1 that in CHO-K1 cells (lane 3). Increment of plasmalogens by addition of Etn was confirmed by TLC analysis (right panel). Lanes 1 and 6, mock-treated CHO-K1 cells. B, CHO-K1 cells were cultured in the absence (lane 1) or presence (lane 2) of Etn for 43 h and further incubated with [14C]acetate for 5 h. Lipids were extracted and separated on TLC as described under “Experimental Procedures.” Squalene, MOS, cholesterol, and 24,25-EC were detected by a FLA-5000 imaging analyzer. Cellular plasmalogens (lower panel) were detected by exposing to iodine vapor. Synthesis of squalene is represented by taking as 1 that in CHO-K1 cells (lower panel). *; p < 0.05; t test versus untreated-CHO-K1 cells. C, HeLa cells were cultured for 2 h in the presence of 20 μM Ro48-8071 (Ro), an inhibitor of lanosterol synthase, or cultured for 43 h in the absence (lane 1) or presence of Etn (lane 2), and incubated for another 5 h for metabolic labeling with [14C]acetate in the presence (lane 3) or absence (lanes 1 and 2) of Ro48-8071. Lipids were extracted and analyzed as in B. Dot indicates an unidentified lipid. Synthesis of squalene is represented by taking as 1 that in HeLa cells (lower panel). *; p < 0.05; t test versus untreated-HeLa cells.

sized via the isoprenoid biosynthetic pathway. If the increased level of plasmalogens suppresses the rate of any steps of isoprenoid synthesis including HMGR, a rate-limiting enzyme of the cholesterol synthesis, farnesylation, and/or geranylgeranylation of proteins such as Pex19p (18, 26) and Rab5 (27) would be reduced as reported (28). Therefore, we verified the isoprenylation of Pex19p, Rab5, and Rab4 (Fig. 3A). When cells were treated withLovastatin, an HMGCR inhibitor, the non-farnesyalted form of Pex19p and non-geranyleranlated form of Rab4 and Rab5 were recovered in the cytosol fraction, each with a slower mobility than the modified form in SDS-PAGE, consistent with a previous report (28). However, these unmodified Pex19p, Rab5, and Rab4 were not observed when plasmalogen levels were elevated in CHO-K1, hence indicating that isoprenoid synthesis was not altered by the elevation of plasmalogens.

We next determined the level of sterol intermediates in the cholesterol biosynthetic pathway. Lanosterol, the first sterol intermediate in cholesterol biosynthesis, is synthesized via condensation of isoprene to squalene and subsequent oxidation of squalene to MOS, followed by cyclization of MOS. 24,25-EC is generated de novo by a shunt of the cholesterol biosynthetic pathway through synthesis of 2,3,22,23-diepoxyqsualene (DOS) from MOS (29). When CHO-K1 cells were metabolically labeled with [14C]acetate, synthesis of MOS, cholesterol, and 24,25-EC was apparently reduced upon elevation of plasmalogens (Fig. 3B). In HeLa cells, MOS, lanosterol, cholesterol, and 24,25-EC were detected at a reduced level upon the elevation of plasmalogens. When lanosterol synthase (LSS) activity was partially inhibited by Ro48-8071, an inhibitor of LSS (30, 31) in HeLa cells, synthesis of lanosterol and cholesterol was lowered, whereas marked accumulation of MOS and slight elevation of 24,25-EC were observed (Fig. 3C), distinct from the results obtained from the elevation of plasmalogens, suggesting that LSS activity is not suppressed upon the elevation of plasmalogens. Moreover, the squalene level was slightly elevated by the increase of plasmalogens in CHO-K1 and HeLa cells (Fig. 3, B and C). Taken together, these results suggest that the oxidation step catalyzed by SQLE is most likely down-regulated by the elevation of plasmalogens.
Elevation of Plasmalogens Causes Degradation of SQLE in a MARCH6-dependent Manner—We next assessed the expression level of SQLE in HeLa cells. Upon elevation of the plasmalogen level, expression of SQLE was reduced to about 50% as compared with the untreated cells, whereas the expression level of P450 reductase (P450R), an endoplasmic reticulum enzyme, was not altered by the elevation of plasmalogens. The reduced SQLE level was fully recovered to that of untreated cells by treatment of the cells with epoxomicin, a proteasomal inhibitor (Fig. 4, A, lane 4). The transcription level of SQLE, LSS, and HMGCR was not altered upon the elevation of plasmalogens (Fig. 4B), where the transcription level of these enzymes was lowered with rapamycin (24). Furthermore, cycloheximide chase experiments using HeLa cells in the presence of Etn revealed that elevation of plasmalogens stimulated degradation of SQLE as compared with the turnover of SQLE in the absence of Etn (Fig. 4C). Taken together, these results suggest that SQLE is specifically degraded upon elevation of plasmalogens in a proteasome-dependent manner.

SQLE is reported to be degraded by membrane-associated RING finger 6 (MARCH6) in a manner dependent on cholesterol (32, 33). Therefore, we investigated whether plasmalogen-dependent degradation of SQLE is also mediated by MARCH6 by expressing MARCH6 or MARCH6C9A, an inactive form of
MARCH6 (Fig. 4D). Expression of MARCH6C9A-Myc was elevated the level of SQLE, whereas the expression of MARCH6-MycB slightly reduced the SQLE level, consistent with the earlier studies (32). The expression level of MARCH6 was lower than MARCH6C9A, most likely due to the degradation mediated by autoubiquitination of wild-type MARCH6 (32, 34). Degradation of SQLE was stimulated by expression of MARCH6-MycB in the presence of Etn, which was partially inhibited by the expression of MARCH6C9A-Myc. Moreover, plasmalogen-dependent degradation of SQLE was interfered by the treatment of dsRNA against MARCH6 (Fig. 4E), where the transcriptional level of MARCH6 was reduced about 50% (data not shown). In addition, turnover of a cholesterol-dependent degradation of SQLE in plasmalogen-elevated cells had a similar tendency to that in the cells cultured in the absence of Etn, where the cellular free cholesterol level was slightly lower in plasmalogen-elevated cells (Fig. 4F). Taken together, these results strongly suggested that SQLE is degraded in a MARCH6-dependent manner upon elevation of plasmalogens.

Cholesterol Synthesis in Plasmalogen-deficient Cells—We next investigated cholesterol synthesis in plasmalogen-deficient cells such as fibroblasts from an ADAPS-deficient patient and an ADAPS-defective CHO mutant, adaps ZPEG251 (6, 13). Cholesterol was more efficiently synthesized than 24,25-EC in human fibroblasts from a normal control (Fig. 5A). In contrast, 24,25-EC was more effectively synthesized than cholesterol in fibroblasts from an ADAPS-deficient patient. In addition, there was the same tendency in the sterol synthesis in plasmalogen-deficient CHO cell mutant, adaps ZPEG251 (Fig. 5B), as indicated in the ratio the 24,25-EC/cholesterol(Fig. 5B, lower panel). These results suggest that the abnormally low level of plasmalogens causes suppression of cholesterol synthesis.

In the cholesterol synthetic pathway, MOS is converted to either cholesterol or further epoxidized to DOS that leads to the synthesis of 24,25-EC (35, 36). Interestingly, MOS was under the detectable level in ZPEG251, which was restored to the normal level in ZPEG251/ADAPS-HA2 cells stably expressing ADAPS-HA2 (Fig. 5, B and C). Fatty alcohols accumulate in plasmalogen-deficient cells (6, 37). Suppression of fatty alcohol synthesis by lowering the FAR1 expression did not restore MOS synthesis, implying that the absence of plasmalogens, but not the accumulation of fatty alcohols, results in the reduction of MOS synthesis (Fig. 5C). Together, SQLE more likely prefers to synthesize DOS in plasmalogen-deficient cells, thereby giving rise to the reduced level of MOS utilized for the synthesis of cholesterol.

To investigate the synthesis of DOS in plasmalogen-deficient cells, plasmalogen-deficient -replete cells were treated with Ro48-8071, an inhibitor of LSS. Synthesis of 24,25-EC was mildly inhibited, whereas synthesis of cholesterol was efficiently suppressed, hence suggesting that Ro48-8071 partially inhibited the LSS activity (31). Upon such treatment, DOS accumulated more in ZPEG251 cells than CHO-K1 and ZPEG251/ADAPS-HA2 cells (Fig. 5D). Taken together, these results suggest that the distinct reduction of cholesterol synthesis in plasmalogen-deficient cells is most likely due to the limited amount of MOS available for the synthesis of cholesterol.

Expression of SQLE Is Elevated in Plasmalogen-deficient Cells—Synthesis of 24,25-EC is modulated by the relative activities of SQLE and LSS (36). Partial inhibition of LSS activity or overexpression of SQLE stimulates 24,25-EC synthesis (31, 38). However, accumulation of DOS was not observed in the absence of Ro48-8071 in ZPEG251 (data not shown), suggesting that the reduced activity of LSS is unlikely for the preferential DOS production in plasmalogen-deficient cells. Therefore, we investigated the expression level of SQLE in plasmalogen-deficient cells including ADAPS-defective fibroblasts and ZPEG251 and found that SQLE was expressed at about a 2-fold

**FIGURE 4. Increase in cellular plasmalogens reduces the levels of SQLE.** A, left panel, HeLa cells were cultured with Etn (lanes 3 and 4) for 43 h and further cultured for 5 h in the presence (lanes 1 and 4) or absence (lanes 2 and 3) of 10 μM epoxomicin (Epoxi), an inhibitor of proteasome. Expression level of SQLE, P540 reductase (P540R), and α-tubulin (α-Tub) was assessed by immunoblotting with antibodies as indicated on the left panel. Upper panel, left fibroblasts from an ADAPS-deficient patient were cultured for 48 h in the presence (lanes 2 and 3) or absence (lanes 1 and 4) of Etn. The expression levels of SQLE and actin were assessed by immunoblotting with antibodies as indicated on the left panel. Lower panel, left fibroblasts from an ADAPS-deficient patient were cultured for 50 h in the presence (lanes 2 and 3) or absence (lanes 1 and 4) of Etn. The expression levels of SQLE and actin were assessed by immunoblotting with antibodies as indicated on the left panel. B, middle panel, the relative expression level of SQLE was represented by taking as 100 that in mock-treated HeLa cells. *, p < 0.05; t test versus SQLE level at the same time in the absence of Etn. Right panel, cellular plasmalogens were detected by LC-ESI-MS/MS and relative plasmalogen levels at the time point of cycloheximide addition were shown. *, p < 0.05; t test versus a control. **, p < 0.01; t test versus Etn. C, left panel, HeLa cells were cultured in the absence (upper panel) or presence (lower panel) of Etn for 40 h and further incubated for 8 h in the presence of cycloheximide (CHX, 100 μg/ml) and assessed for the expression of SQLE at each time point. Middle panel, SQLE bands at each time point were quantified. Relative amounts of SQLE in the presence (square) or absence (triangle) of Etn at each time point were represented by taking as 100 that at the time point of cycloheximide addition. *, p < 0.05; t test versus SQLE level at the same time in the absence of Etn. CHX, 100 μg/ml.
Plasmalogens Regulate Cholesterol Biosynthesis

FIGURE 5. Synthesis of sterols in plasmalogen-deficient cells. A, fibroblasts derived from a healthy control (Cont.) and a patient defective in ADAPS (adaps) were metabolically labeled for 5 h with \[^{14}C\]acetate and assessed for sterol synthesis. Origin indicates the spots where the extracted lipids were placed on TLC (upper panel). Synthesis levels of 24,25-EC and cholesterol in control (solid bar) and adaps-deficient (open bar) fibroblasts were shown (lower panel). *, p < 0.01; t test versus control fibroblasts. B, synthesis of MOS (upper panel) and sterols (middle panel) in CHO-K1, adaps ZPEG251, and ZPEG251/ADAPS-HA2 were analyzed. Note that synthesis of cholesterol and MOS was specifically abrogated in ZPEG251. The ratio of 24,25-EC to cholesterol is presented (lower panel), *, p < 0.01; t test versus CHO-K1, **, p < 0.01; t test versus ZPEG251. C, ZPEG251/ADAPS-HA2, ZPEG251, and ZPEG251 that had been treated with double-strand RNA against FAR1 were metabolically labeled with \[^{14}C\]acetate and analyzed by autoradiography for synthesis of MOS (upper panel) and accumulation of fatty alcohol (middle panel). Relative amount of MOS was represented by taking as 100 that in ZPEG251/ADAPS-HA2 (lower panel), *, p < 0.01; t test versus ZPEG251/ADAPS-HA2. D, CHO-K1, ZPEG251, and ZPEG251/ADAPS-HA2 were treated with 20 \(\mu\)M Ro48-8071 for 2 h and metabolically labeled with \[^{14}C\]acetate in the presence of the inhibitor. Synthesis of DOS (upper panel) and sterols (middle panel) was analyzed. Relative amount of DOS was represented by taking as 100 that in CHO-K1 (lower panel), *, p < 0.01; t test versus CHO-K1, **, p < 0.01; t test versus ZPEG251.

higher level in plasmalogen-deficient cells than that in control fibroblasts and CHO-K1 cells (Fig. 6, A and B). Quantitative RT-PCR analysis showed that the mRNA expression level of SQLE in plasmalogen-deficient cells was not altered as compared with that in control cells (Fig. 6, A and B, lower panels). Furthermore, cycloheximide chase experiments revealed that SQLE in plasmalogen-deficient fibroblasts was more stable than that in control cells (Fig. 6C). Collectively, these results suggest that SQLE is stabilized in plasmalogen-deficient cells more likely by a post-translational mechanism.

SQLE Interacts with MARCH6 in a Plasmalogen-dependent Manner—Knockdown of MARCH6 stabilizes SQLE under normal culture conditions and abolishes cholesterol-dependent degradation of SQLE (32, 33). Therefore, we investigated if the absence of plasmalogens results in degradation or inactivation of MARCH6, giving rise to the elevation of the expressed levels of SQLE. However, addition of cholesterol in wild-type CHO-K1 and ZPEG251 similarly promoted degradation of SQLE (Fig. 7A), suggesting that expression and activity of MARCH6 was not altered in the absence of plasmalogens.

We further assessed whether plasmalogens regulate interaction of SQLE with MARCH6 by a coimmunoprecipitation assay. HA-tagged SQLE (SQLE-HA2) plus MARCH6-Myc or MARCH6C9A-Myc were coexpressed in ZPEG251 and CHO-K1 cells, and subjected to immunoprecipitation using anti-Myc antibody. SQLE-HA2 was coimmunoprecipitated with MARCH6C9A-Myc in ZPEG251 and CHO-K1 (Fig. 7C), consistent with the earlier study (32). Moreover, a larger amount of SQLE-HA2 was coimmunoprecipitated with MARCH6C9A-Myc in ZPEG251 and CHO-K1, hence suggesting that plasmalogens regulate interaction of MARCH6-Myc upon the elevation of plasmalogens in CHO-K1 cells. Similarly, the elevation of plasmalogens stimulates the interaction of MARCH6-Myc with SQLE-HA2 (Fig. 7B), where the interaction of SQLE-HA2 with MARCH6-Myc in CHO-K1 and ZPEG251 was less efficient. Together, these results suggest that plasmalogens regulate the stability of SQLE by modulating interaction of SQLE with MARCH6.

Discussion

In the present study, we show that the cellular plasmalogen level regulates cholesterol synthesis by modulating SQLE stability. Cholesterol synthesis is shown to be controlled at multiple steps, including sterol regulatory element-binding protein (SREBP)-mediated transcriptional regulation and post-translational regulation of HMGR, a rate-limiting enzyme of cholesterol synthesis (39, 40). In addition, recent studies revealed that the activity of SQLE is controlled at a post-translational level through the cholesterol-dependent ubiquitination and protosomal degradation (12, 32, 33). In the present study, our finding that elevation of cellular plasmalogens also causes suppression of cholesterol synthesis in several cell lines (Figs. 1 and 2), implies that plasmalogen-dependent degradation of SQLE is a conserved mechanism for the regulation of cholesterol synthesis.

MARCH6 is an E3 ligase responsible for the degradation of SQLE in response to an exogenous cholesterol influx (32, 33). SQLE expression is elevated by reduction of MARCH6 (32, 33) (Fig. 4E), expression of an inactive form of MARCH6 (33) (Fig. 4D), or treatment of cells with epoxymycin (Fig. 4A), hence suggesting that SQLE is constitutively degraded in a MARCH6-dependent manner. Indeed, SQLE is constitutively degraded in SRD-1 cells (32), HeLa (Fig. 4C), and normal control fibroblasts (Fig. 6C). Moreover, SQLE interacts with MARCH6 without addition of external cholesterol (Fig. 7B) (32), where the interaction is increased upon elevation of cellular plasmalogens. Taken together, we suspect that the constitutive degradation of SQLE is more likely stimulated by the elevation of plasmalogens, but not cholesterol. Exogenous supplementation of cholesterol to the culture cells induces degradation of SQLE (12, 32,
33) by enhancing MARCH6-mediated ubiquitination (32). However, cholesterol-dependent degradation of SQLE in plasmalogens-deficient cells was likewise detected as that in wild-type cells (Fig. 7A). Moreover, elevation of plasmalogens did not synergistically augment the cholesterol-dependent degradation of SQLE in HeLa cells (Fig. 4F). Accordingly, we suggest that plasmalogens augment the interaction of SQLE with MARCH6 by a mechanism distinct from that involving cholesterol, although the mechanism underlying cholesterol-dependent interaction between SQLE and MARCH6 is not defined.

In humans, MARCH6 is expressed in several different tissues including heart, brain, kidney, and liver (41). However, only three proteins, including SQLE, HMGCR, and type 2 iodothyronine deiodinase, have been so far identified as a substrate for MARCH6. Therefore, the full range of cellular cargoes for MARCH6 remains to be determined.

FIGURE 6. Expression level of SQLE is elevated in the absence of plasmalogens. A, expression level of SQLE was assessed by immunoblotting in fibroblasts derived from a control (Cont.) and a patient defective in ADAPS (adaps) (upper panel). Actin was used as a loading control. Relative expression levels of SQLE were represented by taking as 100 that in control fibroblasts (middle panel). *, p < 0.05; t test versus control. Transcription levels of SQLE and LSS in control (Cont., solid bar) and ADAPS-defective fibroblasts (adaps, open bar) were assessed by real-time PCR (lower panel). Relative expression levels of SQLE and LSS are represented by taking that as 100 in control fibroblasts. B, expression level of SQLE was assessed in CHO-K1, ZPEG251, and ZPEG251/ADAPS-HA2 (upper panel). Tubulin (α-Tub) was used as a loading control. The relative expression level of SQLE was represented by taking as 100 that in CHO-K1 (middle panel). *, p < 0.01; t test versus CHO-K1. **, p < 0.01; t test versus ZPEG251. Relative expression levels of SQLE and LSS to the housekeeping gene PBGD (porphobilinogen deaminase) (12, 23) was analyzed by real-time PCR using total RNA prepared from CHO-K1 (solid bar), adaps ZPEG251 (gray bar), and ZPEG251/ADAPS-HA2 (open bar) (lower panel). Relative expression levels of SQLE and LSS are represented by taking that as 100 in CHO-K1 cells (n = 3). C, SQLE is more stable in adaps fibroblasts. Control (Cont.) and ADAPS-defective (adaps) fibroblasts were cultured for 9 h in the presence of cycloheximide (100 μg/ml) and assessed for the expression level of SQLE at each time point (left panel). Relative amounts of SQLE in control (triangle) and ADAPS-defective (square) fibroblasts at each time point were represented by taking as 100 that at the time point of cycloheximide addition (right panel).
MARCH6-mediated degradation in mammals (32, 33, 42). However, MARCH6-mediated degradation of HMGCR seems to be independent of plasmalogens because isoprenylation of Pex19p, Rab5, and Rab4 (Fig. 3A), and squalene synthesis (Fig. 3, B and C) were not reduced by the increment of plasmalogens. Further studies including identification of more potential substrates of MARCH6 and the effects of plasmalogens on the degradation of such substrates are clearly required.

Sterol-dependent degradation of SQLE seems to be a conserved mechanism in higher eukaryotes (43). Sterol-dependent degradation of ERG1, the SQLE homologue, is likewise mediated by MARCH6 homologue Doa10 in Saccharomyces cerevisiae (33). A similar mechanism was postulated (32, 44) from the findings that accumulation of squalene in the SQLE-defective plant was restored when a mutant allele of the plant homologue of MARCH6, SUD1, was crossed into this background (32, 44). However, plasmalogens are not synthesized in plant (2, 45), whereas only trace amounts of plasmalogens are detected in S. cerevisiae (46). Given these findings, we suggest that plasmalogen-mediated degradation of SQLE is specific for mammals. SQLE apparently spans the endoplasmic reticulum membrane via its hydrophobic segment located in the N-terminal region (12, 47), although the membrane topology of SQLE is not yet defined. Interestingly, the region encompassing the 100-amino acid sequence including a potential transmembrane domain of human SQLE is sufficient for cholesterol-dependent degradation (12). However, this region is absent from yeast ERG1, and only a few amino acids are conserved between plant and human

**FIGURE 7.** Plasmalogens modulate the interaction of SQLE with MARCH6. A, CHO-K1 and adaps ZPEG251 were cultured for 16 h in the presence of cholesterol (20 μg/ml) and assessed for the expression level of SQLE by immunoblotting (left panel). Tubulin (α-Tub) was used as a loading control. Dot indicates a truncated form of SQLE. Cellular free cholesterol was detected with iodine vapor (left panel) and by the enzymatic method (Table 1). B, upper panel, SQLE-HA2 was coexpressed with mock vector (lanes 7 and 8) or MARCH6–MycC (lanes 1–6), cultured for 43 h in the presence of cholesterol (lanes 1 and 4) or absence (lanes 2, 3, and 5–8) of Etn, and further cultured for 5 h in the presence of 10 μM epoxomycin. Cell lysates were subjected to coimmunoprecipitation (IP) using rabbit anti-Myc antibody as indicated at the top. MARCH6–MycC and SQLE–HA2 were detected with monoclonal antibodies to Myc and HA tags, respectively. Input (In.), 1% of cell lysates used for immunoprecipitation. Lower panel, coimmunoprecipitation of MARCH6–MycC with SQLE–HA2 was assessed and represented as a ratio of SQLE–HA2 versus MARCH6–MycC, by taking as 1 that in CHO-K1 treated with Etn. **, p < 0.01; t test versus Etn. C, upper panel, SQLE-HA2 was coexpressed with mock vector (lanes 1 and 2) or MARCH6C9A–MycC (lanes 3–8) in CHO-K1 (lanes 1–4, 6, and 7) and adaps ZPEG251 (lanes 5 and 8), and cultured for 2 days in the presence (lanes 3 and 6) or absence (lanes 1, 2, 4, 5, 7, and 8) of Etn. Input (In.), 1% of cell lysates used for immunoprecipitation. Lower panel, interaction of MARCH6C9A–MycC with SQLE–HA2 was verified as in B. *, p < 0.05; t test versus Etn. **, p < 0.01; t test versus Etn.
SQLE. Accordingly, we propose that plasmalogens affect the interaction of SQLE with MARCH6 via the transmembrane domain in the N-terminal region of SQLE.

SQLE is proposed as the second rate-limiting enzyme in cholesterol synthesis (10–12). Inhibition of SQLE efficiently reduces cholesterol synthesis (48, 49), where accumulation of squalene does not cause any major adverse effects (50). Furthermore, the reduced level of cholesterol synthesis in plasmalogen-elevated cells is consistent with the lowered level of HMGCR activity (51) and cholesterol synthesis (12) in the cholesterol-elevated cells. Therefore, suppression of SQLE expression by elevation of plasmalogens might be an alternative potential way to reduce cholesterol synthesis without affecting the synthesis of physiologically important metabolites in the mevalonate pathway, including dolichol, ubiquinone, heme A, and prenylated proteins.

In the present study, we found that synthesis of cholesterol was specifically reduced in plasmalogen-deficient cells such as adaps CHO mutant ZPEG251, whereas 24,25-EC synthesis was elevated in plasmalogen-deficient ZPEG251 and fibroblasts derived from an ADAPS-deficient patient when cultured in the presence of FCS (Fig. 5). Our result is not compatible with that of the earlier study addressing newly synthesized cholesterol in the presence of lipoprotein-deficient serum (52). The HMGCR activity (51) and synthesis of cholesterol (52) are dramatically increased in the presence of lipoprotein-deficient serum, suggesting that the ablation of cholesterol synthesis in plasmalogen-deficient cells is hindered by stimulating mevalonate synthesis. Interestingly, endoplasmic reticulum stress in the PEX2−/− mouse liver causes an elevation of cholesterol synthesis via activation of the SREBP-2 pathway (53–55), whereas cholesterol synthesis in the PEX5−/− mouse is not affected (56). Transcription of SQLE and LSS, targets in the SREBP-2 pathway (36, 57), were not elevated in adaps ZPEG251 and fibroblasts derived from a patient defective in ADAPS (Fig. 6, A and B), suggesting that elevation of cholesterol synthesis in the PEX2−/− mouse liver is caused by multiple peroxisomal dysfunctions as well as the defect in plasmalogen synthesis.

Expression of SQLE is at a very low level in most non-cholesterogenic tissues, whereas SQLE is highly abundant in liver, followed by gut, skin, and neural tissue (58). Interestingly, the cellular plasmalogen level is very low in several tissues such as liver and small intestine (59). Therefore, the limited amount of plasmalogens in liver may contribute to the high level of SQLE expression by suppressing the MARCH6-mediated degradation of SQLE, thereby resulting in the efficient synthesis of 24,25-EC as observed in plasmalogen-deficient cells (Fig. 5).

Synthesis of 24,25-EC is regulated by the relative activity of SQLE and LSS (36). LSS activity is high in cholesterogenic and non-cholesterogenic tissues (58). Therefore, it is likely that synthesis of 24,25-EC is dependent on the activity of SQLE. However, the regulation mechanism of SQLE activity remains unknown. Synthesis of DOS and 24,25-EC is elevated in plasmalogen-deficient cells (Fig. 5) and 24,25-EC synthesis is increased by overexpression of SQLE (60). Taken together, the synthesis rate of 24,25-EC more likely depends on the expression level of SQLE, although we cannot exclude the possibility that unidentified post-translational modification of SQLE contributes to the regulation of SQLE activity.

24,25-EC is a physiological ligand for liver X receptor (LXR) in liver (61), playing a role in the reverse transport of cholesterol by stimulating transcription of ABCA1 encoding ATP-binding cassette transporter A1 (62–65) and IDOL coding for the inducible degrader of the LDL receptor (66). Because 24,25-EC synthesis is elevated in plasmalogen-deficient cells, we suspect that a limited amount of plasmalogens in liver manipulates the synthesis of 24,25-EC toward the effective reverse cholesterol transport. Plasmalogen deficiency causes the impaired high-density lipoprotein (HDL)-mediated cholesterol efflux from murine macrophage-like cells (67), the altered transport of internalized cholesterol to the endoplasmic reticulum (3), and abnormal cellular distribution of cholesterol (68). Moreover, addition of cis-(-)-2-O-docosahexaenoyl-1-O-hexadecylglycerol increases cholesterol esterification by raising the sterol-O-acyltransferase 1 expression level in HEK293 cells (69) and elevation of plasmalogens reduces the cholesterol level in HeLa cells cultured in the presence of exogenously added cholesterol (Fig. 4F). Taken these together with our findings, we suggest that the cellular plasmalogen level plays an important role at multiple steps in cholesterol homeostasis.

Plasmalogens are the major glycerophospholipids in brain and nerve tissue (70–72). Cholesterol, which is required for the proper function of neuronal cells, is mainly provided by glial cells in the brain, because the blood-brain barrier separates the brain from the circulating cholesterol (73). Therefore, the regulation of plasmalogen synthesis most likely plays a pivotal role in the homeostasis of cholesterol especially in the central nervous system. Neurons do not efficiently synthesize cholesterol and mainly take up the cholesterol produced by astrocytes (74), hence suggesting that regulation of plasmalogen homeostasis unequivocally contributes to regulation of cholesterol synthesis in astrocytes. Our findings reported here open a way to address cholesterol homeostasis involving plasmalogen physiology.

**Author Contributions**—M. H. designed the research and performed experiments. Y. A. performed the LC-MS/MS analysis. M. H. and Y. F. analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

**Acknowledgments**—We thank Y. Nanri and S. Okuno for technical assistance, K. Shimizu for preparing figures, and the other members of our laboratory for discussions.

**References**

1. Steinberg, S. J., Dodt, G., Raymont, G. V., Braverman, N. E., Moser, A. B., and Moser, H. W. (2006) Peroxisome biogenesis disorders. *Biochim. Biophys. Acta* 1763, 1733–1748
2. Braverman, N. E., and Moser, A. B. (2012) Functions of plasmalogen lipids in health and disease. *Biochim. Biophys. Acta* 1822, 1442–1452
3. Munn, N. J., Arnio, E., Liu, D., Zoeller, R. A., and Liscum, L. (2003) Deficiency in ethanolamine plasmalogen leads to altered cholesterol transport. *J. Lipid Res.* 44, 182–192
4. Teigler, A., Komljenovic, D., Draughn, A., Gorgas, K., and Just, W. W. (2009) Defects in myelination, paranode organization and Purkinje cell innervation in the ether lipid-deficient mouse cerebellum. *Hum. Mol. Genet.* 18, 1897–1908
5. Cheng, J. B., and Russell, D. W. (2004) Mammalian wax biosynthesis. I.
Plasmalogens Regulate Cholesterol Biosynthesis

identification of two fatty acyl-coenzyme a reductases with different substrates specificity and tissue distributions. J. Biol. Chem. 279, 37789–37797

6. Honsjo, M., Asaoku, S., and Fujiki, Y. (2010) Posttranslational regulation of fatty acyl-CoA reductase 1, Far1, controls ether glycerophospholipid synthesis. J. Biol. Chem. 285, 8537–8542

7. Honsjo, M., Asaoku, S., Fukumoto, K., and Fujiki, Y. (2013) Topogenesis and homeostasis of fatty acyl-CoA reductase 1. J. Biol. Chem. 288, 34588–34598

8. Buchert, R., Tawamie, H., Smith, C., Uebe, S., Innes, A. M., Al Hallak, B., Ekici, A. B., Sticht, H., Schwarze, B., Lamont, R. E., Parboosingh, J. S., Bernier, F. P., and Abou Jamra, R. (2014) A peroxisomal disorder of severe intellectual disability, epilepsy, and cataracts due to fatty acyl-CoA reductase 1 deficiency. Am. J. Hum. Genet. 95, 602–610

9. Burg, J. S., and Espenshade, P. J. (2011) Regulation of HMG-CoA reductase in mammary and yeast. Prog. Lipid Res. 50, 403–410

10. Hidaka, Y., Sato, T., and Kamei, T. (1990) Regulation of squalene epoxidase in HepG2 cells. J. Lipid Res. 31, 2067–2094

11. Gonzalez, R., Carlson, J. P., and Dempsey, M. E. (1979) Two major regulatory steps in cholesterol synthesis in human renal cancer cells. Arch. Biochem. Biophys. 196, 574–580

12. Gill, S., Stevenson, J., Kristiana, I., and Brown, A. J. (2011) Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. Cell Metab. 13, 260–273

13. Honsjo, M., Yagita, Y., Kinoshita, N., and Fujiki, Y. (2008) Isolation and characterization of mutant animal cell line defective in alkyl-dihydroxyacetonephosphate synthase: Localization and transport of plasmalogens to post-Golgi compartments. Biochim. Biophys. Acta 1783, 1857–1865

14. Okumoto, K., Bogaki, A., Tateishi, K., Tsukamoto, T., Osumi, T., Shimozawa, N., Suzuki, Y., Orii, T., and Fujiki, Y. (1997) Isolation and characterization of peroxisome-deficient Chinese hamster ovary cell mutants representing human complementation group III. Exp. Cell Res. 233, 11–20

15. Klein, U., Gimpil, G., and Fahrenholz, F. (1995) Alteration of the myome

sterol biosynthetic gene expression and exhibits a rapamycin-resistant transcriptional profile. Proc. Natl. Acad. Sci. U.S.A. 108, 15201–15206

25. Du, X., Kristiana, I., Wong, J., and Brown, A. J. (2006) Involvement of Akt in ER-to-Golgi transport of SCAP/SREBP: a link between a key cell proliferative pathway and membrane synthesis. Mol. Biol. Cell 17, 2735–2745

26. James, G. L., Goldstein, J. L., Pathak, R. K., Anderson, R. G. W., and Brown, M. S. (1994) Peroxisome proliferator of peroxisomes. J. Biol. Chem. 269, 14182–14190

27. Kinlessa, B. T., and Maltese, W. A. (1992) Rab GTP-binding proteins with three different carboxyl-terminal cysteine motifs are modified in vivo by 20-carbon isoprenoids. J. Biol. Chem. 267, 3940–3945

28. Ostrowski, S. M., Wilkinson, B. L., Golde, T. E., and Landreth, G. (2007) Statins reduce amyloid-β production through inhibition of protein iso

prenylation. J. Biol. Chem. 282, 26832–26844

29. Gill, S., Chow, R., and Brown, A. J. (2008) Sterol regulators of cholesterol homeostasis and beyond: the oxysterol hypothesis revisited and revised. Prog. Lipid Res. 47, 391–404

30. Morand, O. H., Aebi, J. D., Dehmlow, H., Ji, Y. H., Gains, N., Lengsfeld, H., and Himber, J. (1997) Ro 48–8071, a new 2,3-oxidosqualene: lanosterol cyclase inhibitor lowering plasma cholesterol in hamsters, squirrel monkeys, and minipigs: comparison to simvastatin. J. Lipid Res. 38, 373–390

31. Dang, H., Liu, Y., Pang, W., Li, C., Wang, N., Shyy, J. Y., and Zhu, Y. (2009) Suppression of 2,3-oxidosqualene cyclase by high fat diet contributes to liver X receptor-α-mediated improvement of hepatic lipid profile. J. Biol. Chem. 284, 6218–6226

32. Zelter, N., Sharpe, L. J., Loregger, A., Kristiana, I., Cook, E. C. L., Phan, L., Stevenson, J., and Brown, A. J. (2014) The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and the cholesterol synthesis pathway. Mol. Cell. Biol. 34, 1262–1270

33. Foresti, O., Ruggiano, A., Hannibal-Bach, H. K., Ejsing, C. S., and Carvalho, P. (2013) Sterol homeostasis requires regulated degradation of squalene monooxygenase by the ubiquitin ligase Dda10/Teb4. eLife 2, e00953

34. Hassink, G., Kikkert, M., van Voorden, S., Lee, S. J., Spaepen, R., van Laar, T., Coleman, C. S., Bartee, E., Früh, K., Chau, V., and Wiertz, E. (2005) TEB4 is a C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum. Biochem. J. 388, 647–655

35. Wong, J., Quinn, C. M., and Brown, A. J. (2007) Synthesis of the oxysterol, 24(S), 25-epoxycholesterol, parallels cholesterol production and may protect against cellular accumulation of newly-synthesized cholesterol. Lipids Health Dis. 6, 10

36. Sharpe, L. J., and Brown, A. J. (2013) Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCGR). J. Biol. Chem. 288, 18707–18715

37. Rizzo, W. B., Craft, D. A., Judd, L. L., Moser, H. W., and Moser, A. B. (1993) Fatty alcohol accumulation in the autosomal recessive form of rhizomelic chondrodysplasia punctata. Biochem. Med. Metab. Biol. 50, 93–102

38. Zerenturk, E. J., Sharpe, L. J., and Brown, A. J. (2012) Sterols regulate 3β-hydroxysterol Δ24-reductase (DHCR24) via dual sterol regulatory elements: cooperative induction of key enzymes in lipid synthesis by sterol regulatory element binding proteins. Biochim. Biophys. Acta 1821, 1350–1360

39. Goldstein, J. L., DeBose-Boyd, R. A., and Brown, M. S. (2006) Protein sensors for membrane sterols. Cell 124, 35–46

40. Sato, R. (2010) Sterol metabolism and SREBP activation. Arch. Biochem. Biophys. 501, 177–181

41. Nagase, T., Ishikawa, K., Miyaijima, N., Tanaka, A., Kotani, H., Nomura, N., and Ohara, O. (1998) Prediction of the coding sequences of unidentified human genes: IX. the complete sequences of 100 new cDNA clones from human brain which can code for large proteins. DNA Res. 5, 31–39

42. Zavacki, A. M., Arrojo E Drigo, R., Freitas, B. C. G., Chung, M., Harney, J. W., Egri, P., Wittmann, G., Fekete, C., Gereben, B., and Bianco, A. C. (2009) The E3 ubiquitin ligase TEB4 mediates degradation of type 2 iodothyronine deiodinase. Mol. Cell. Biol. 29, 5339–5347

43. Sharpe, L. J., Cook, E. C. L., Zelter, N., and Brown, A. J. (2014) The UPS and down of cholesterol homeostasis. Trends Biochem. Sci. 39, 527–535

44. Doblas, V. G., Amorim-Silva, V., Posé, D., Rosado, A., Esteban, A., Arró, M., Azavedo, H., Bombarely, A., Borsani, O., Valpuesta, V., Ferrer, A.,
Plasmalogens Regulate Cholesterol Biosynthesis

H. H. M., and Tontonoz (2014) Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat. Rev. Drug Discov.* 13, 433–444

Chen, W., Chen, G., Head, D. L., Mangeldorff, D. J., and Russell, D. W. (2007) Enzymatic reduction of oxysterols impairs LXR signaling in cultured cells and the livers of mice. *Cell Metab.* 5, 73–79

Zelcer, N., Hong, C., Boyadjian, R., and Tontonoz, P. (2009) LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. *Science* 325, 100–104

Mandel, H., Sharf, R., Berant, M., Wanders, R. J. A., Vreken, P., and Aviram, M. (1998) Plasmalogen phospholipids are involved in HDL-mediated cholesterol efflux: insights from investigations with plasmalogen-deficient cells. *Biochem. Biophys. Res. Commun.* 250, 369–373

Thai, T. P., Rodemel, C., Jauch, A., Hunziker, A., Moser, A., Gorgas, K., and Just, W. W. (2001) Impaired membrane traffic in defective ether lipid biosynthesis. *Hum. Mol. Genet.* 10, 127–136

Mankidy, R., Aihiahonu, P. W., Ma, H., Jayasinge, D., Ritchie, S. A., Khan, M. A., Su-Myat, K. K., Wood, P. L., and Goodenowe, D. B. (2010) Membrane plasmalogen composition and cellular cholesterol regulation: a structure activity study. *Lipids Health Dis.* 9, 62

Wanders, R. J. A., and Waterham, H. R. (2006) Biochemistry of mammalian peroxisomes revisited. *Annu. Rev. Biochem.* 75, 295–332

Balakrishnan, S., Goodwin, H., and Cumings, J. N. (1961) The distribution of phosphorus-containing lipid compounds in the human brain. *J. Neurochem.* 8, 276–284

Han, X., Holtzman, D. M., and McKeel, D. W., Jr. (2001) Plasmalogen deficiency in early Alzheimer’s disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. *J. Neurochem.* 77, 1168–1180

Hayashi, H. (2011) Lipid metabolism and glial lipoproteins in the central nervous system. *Bioll. Pharm. Bull.* 34, 453–461

Nieweg, K., Schaller, H., and Pfitzner, F. W. (2009) Marked differences in cholesterol synthesis between neurons and glial cells from postnatal rats. *J. Neurochem.* 109, 125–134

Sakakibara, J., Watanabe, R., Kanai, Y., and Ono, T. (1995) Molecular cloning and expression of rat squalene epoxidase. *J. Biol. Chem.* 270, 17–20