Effects of 25-Hydroxycholesterol on Cholesterol Esterification and Sterol Regulatory Element-binding Protein Processing Are Dissociable

IMPLICATIONS FOR CHOLESTEROL MOVEMENT TO THE REGULATORY POOL IN THE ENDOPLASMIC RETICULUM*

Received for publication, July 30, 2004, and in revised form, August 17, 2004
Published, JBC Papers in Press, August 17, 2004, DOI 10.1074/jbc.M408690200

Ximing Du†, Yen H. Pham‡, and Andrew J. Brown§
From the School of Biotechnology and Biomolecular Sciences, Biological Sciences Building D26, University of New South Wales, Sydney, 2052, Australia

The regulatory pool of cholesterol is located in the endoplasmic reticulum (ER) and is key to how mammalian cells sense and respond to changes in cellular cholesterol levels. The extent of cholesterol esterification by the ER-resident protein, acyl-coenzyme A:cholesterol acyl-transferase (ACAT), has become the standard method for monitoring cholesterol transport to the ER and is assumed to reflect the regulatory pool of ER cholesterol. The oxysterol, 25-hydroxycholesterol (25HC), is thought to trigger intracellular cholesterol transport to the ER. In support of this contention, we confirmed previous reports that 25HC activates cholesterol esterification and is a potent suppressor of the sterol regulatory element-binding protein (SREBP) pathway. Processing of the ER membrane-bound SREBP into a soluble transcription factor is controlled by cholesterol levels in the ER. In this study, we addressed whether or not cholesterol esterification necessarily reflects cholesterol movement to the cholesterol homeostatic machinery in the ER as determined by SREBP processing. We found that three agents that inhibited the ability of 25HC to induce cholesterol esterification (progestosterone, nigericin, and monensin) did not have a corresponding effect on 25HC suppression of SREBP processing. Moreover, ACAT inhibition did not alter the sensitivity of SREBP processing to 25HC. Therefore, cholesterol esterification by the ER-resident protein ACAT is dissociable from cholesterol transport to the cholesterol homeostatic machinery in the ER. In light of our results, we question the security of previous work that has inferred cholesterol transport to the ER regulatory pool based solely on cholesterol esterification.

Although cholesterol is essential in mammalian cells, excess unesterified cholesterol is toxic; therefore, cells maintain their concentrations within narrow limits. Low levels of cholesterol are sensed in the endoplasmic reticulum (ER), and this information is transduced into the expression of multiple homeostatic genes (1–3). Key to this system is a family of membrane-bound transcription factors, sterol regulatory element-binding proteins (SREBPs), which control enzyme levels in cholesterol synthesis, such as 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, as well as the synthesis of low density lipoprotein (LDL)-receptors, which mediate endocytosis of cholesterol-rich LDL particles (4).

Newly synthesized SREBPs form a complex in the ER with the polytopic membrane protein, SREBP cleavage activating protein (SCAP). SCAP serves as both sterol sensor and escort protein for SREBP. When cellular cholesterol levels are sufficient, the SREBP-SCAP complex is tethered in the ER through interaction of SCAP with a retention protein, INSIG-1 (5) or INSIG-2 (6). When cholesterol levels fall, SCAP adopts a conformation that allows dissociation from INSIG proteins and allows the SREBP-SCAP complex to bud from the ER and move to the Golgi via COPII vesicles (7–9). In the Golgi, SREBP is cleaved sequentially by site-1-protease and site-2-protease. This cleavage event releases the soluble amino-terminal domain of SREBP, which then can enter the nucleus as an active, positive transcription factor. Negative feedback regulation by sterols occurs at the level of SCAP-mediated exit of SREBP from the ER (reviewed in Ref. 4).

The ER is home to the principal effectors of cellular cholesterol homeostasis (10). In addition to SCAP, INSIGs, and SREBPs, these include 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase and acyl-coenzyme A:cholesterol acyl-transferase (ACAT). ACAT catalyzes the esterification of cholesterol, and 25-hydroxycholesterol (25HC), 24(S),25-epoxycholesterol, and 27-hydroxycholesterol. These sterols are far more potent suppressors of cholesterol esterification than exercise levels of other cholesterol esterification enzymes, such as 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, which mediate endocytosis of cholesterol-rich LDL particles (4).

* This work was supported by National Heart Foundation of Australia Grant G00S1178. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Both authors contributed equally to this work.
‡ To whom correspondence should be addressed. Tel.: 61-2-9385-2005; Fax: 61-2-9385-1463; E-mail: aj.brown@unsw.edu.au.
§ The abbreviations used are: ER, endoplasmic reticulum; 25HC, 25-hydroxycholesterol; ACAT, acyl-coenzyme A:cholesterol acyl-transferase; CHO-K1, Chinese hamster ovary K1 cells; INSIG, insulin-induced gene; LDL, low density lipoprotein; NBS, newborn calf serum; PBS, phosphate-buffered saline; PLAP, placental alkaline phosphatase; SCAP, SREBP-cleavage activating protein; SREBP, sterol-regulatory element binding protein; CMV, cytomegalovirus.
SREBP processing when added to intact cells than cholesterol (15, 16) and suppress cholesterol synthesis when their endogenous levels have been increased through pharmacologic or genetic manipulation (16–18). Interestingly, these oxysterols are not sensed directly by SCAP as SCAP undergoes a conformational change in response to cholesterol but not these side-chain oxygenated sterols (7). Consistent with these observations, we proposed that these oxysterols may inhibit SREBP processing by causing intracellular cholesterol to the ER, where cholesterol produces a conformational change in SCAP (7). The idea that oxysterols like 25HC trigger movement of cholesterol to the ER is based on the well-documented ability of 25HC to increase cholesterol esterification (13, 19–21).

In this study, we addressed whether or not cholesterol esterification by the ER-resident protein ACAT necessarily reflects cholesterol movement to the cholesterol homeostatic machinery in the ER as determined by SREBP processing. We employed 25HC, a commonly used model oxysterol, to elicit cholesterol transport, and a number of pharmacologic agents that inhibit 25HC-induced cholesterol esterification.

**EXPERIMENTAL PROCEDURES**

**Materials—**All solvents were analytical reagent- or high performance liquid chromatography-grade (Cron Scientific). Dulbecco's modified Eagle's medium/Ham's F-12 medium (1:1 mixture) was obtained from JRH Biosciences. L-Glutamine, newborn calf serum (NBS), and penicillin-streptomycin were obtained from Invitrogen. Lipoprotein-deficient newborn calf serum was prepared as described previously (22). Cholesterol, compactin, n-dithiothreitol, 2-hydroxypropyl-β-cyclodextrin, methyl-β-cyclodextrin, mevalonate, monensin, nigericin, oleic-acoenzyme A, and Sandoz 58-035 were obtained from Sigma. 25-Hydroxycholesterol (cholesterol-5-en-3,25-diol; 25HC) and progesterone were obtained from Steroids. [4α,25-3H]Cholesterol (141.0 Ci/mmol) and [1-14C]oleoyl-coenzyme A (56 mCi/mmol) were obtained from Amersham Biosciences. Antibodies against SCAP and SREBP were kind gifts of Drs. Michael S. Brown and Joseph L. Goldstein: IgG-R139, a rabbit polyclonal antibody against hamster SCAP (amino acids 54–277 and 546–707) (23); IgG-TD4, a mouse monoclonal antibody against hamster SREBP-2 (amino acids 32–250) (24). Mammalian expression plasmids, also generously provided by Drs. Brown and Goldstein, have been described previously: pCMV-PLAP-BP2 (513–1141) (25), pGFP-SCAP (26), pCMV-INSIG-1-Myc (5). LY295427 and LY306039 were kindly donated by Eli Lily (Greenfield, IN).

**Cell Culture—**Chinese hamster ovary cells K1 (CHO-K1) were grown in monolayer in a humidified incubator at 37 °C with 5% CO₂ atmosphere. The cells were grown in 1.1 (v/v) Dulbecco's modified Eagle's medium/Ham's F-12 containing penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM), supplemented with various sera. The different media used were supplemented as follows: Medium A, 10% (v/v) NBS; Medium B, 5% NBS; Medium C, 5% lipoprotein deficient NBS; Medium D, 5% lipoprotein-deficient NBS plus 5 μM compactin and 50 μM mevalonate. Compactin, mevalonate, sterols, and other test agents were added in ethanol. Within an experiment, the final ethanol concentration was kept constant between conditions and did not exceed 0.25% v/v.

**Cholesterol Esterification—**The effect of different compounds on cholesterol esterification was tested using a method described by Lange and colleagues with minor modifications (10). On day 0, CHO-K1 cells were set up (in triplicate) at a density of 4 × 10⁶ cells/60-mm dish in medium A. On day 1, cells were switched to medium C and incubated for 16 h. On day 2, cells were pulse-labeled with [3H]cholesterol complexed to 2-hydroxypropyl-β-cyclodextrin (10 min, 4 °C). After washing with a 0.5 mg/ml solution of bovine serum albumin in phosphate-buffered saline (PBS) (3 × 1 ml, 4 °C), cells were incubated with medium C plus test agents (4 h, 37 °C). Cells were washed twice with PBS and lysed with 0.1 M NaOH (1 ml). Cell lysate was neutralized by 0.1 M HCl. Methanol was added to cell lysate, and lipids were extracted with hexane. Hexane was evaporated, and samples were redissolved in 60 μl of hexane for thin layer chromatography (TLC). [3H]Cholesterol ester and [3H]cholesterol were separated on Silica Gel 60 F₂₅₄ TLC plates (Merck) using heptane:diethyl ether:glacial acetic acid (90:30:1, v/v). Bands, visualized with iodine, were excised and eluted from the silica using methanol (1 ml) followed by diethyl ether (1 ml) with vortex mixing. Radioactivity was measured by β-scintillation counting (Packard Tri-Carb 2100TR Liquid Scintillation Analyzer) using Ultima Gold scintillation liquid (5 ml). Cholesterol esterification is expressed as [3H]cholesteryl ester as a percentage of total radioactivity ([3H]cholesterol plus [3H]cholesterol ester). In some experiments with ACAT inhibition, cells were incubated for 16 h with medium D containing [3H]cholesterol (0.4 μCi/well added in ethanol), and the indicated concentrations of 25HC in the presence or absence of the ACAT inhibitor, Sandoz 58-035 (0.5 μg/ml). Cells were washed and harvested, lipids were extracted, and the extent of cholesterol esterification was determined as described above.

**ACAT Enzyme Activity—**On day 0, CHO-K1 cells were plated at a density of 10⁶ cells/100-mm dish in medium A (7 dishes per condition). On day 1, cells were switched to medium C and incubated for 16 h. On day 2, cells were incubated in medium C in the absence and presence of progesterone (10 μM) for 4 h. Cells were washed with PBS, harvested, and resuspended in 1 ml of Buffer A (10 mM HEPES-KOH at pH 7.4, 10 mM KCl, 1.5 mM MgCl₂, 5 mM sodium EDTA, 5 mM sodium EGTA, and 250 mM sucrose). Cells were lysed by passing through a syringe needle (22 gauge, 30 times), and then centrifuged (1,000 × g, 5 min, 4 °C). The post-nuclear supernatant was centrifuged (20,800 × g, 20 min, 4 °C). The pellet was resuspended in Buffer B (0.1 M Tris-HCl at pH 7.5, 250 mM sucrose containing 1 mg/ml bovine serum albumin). Microsomal protein was determined using the bicinchoninic acid protein assay kit (Pierce). ACAT activity in microsomal fractions was determined by the method of Billheimer and colleagues (27) with some modifications. The reaction contained 200 μg of microsomal protein in Buffer B plus 1 mM diethiothreitol, 20 μg of cholesterol complexed to methyl-β-cyclodextrin

![FIG. 1. 25HC stimulates cholesterol esterification and suppresses SREBP processing in CHO-K1 cells.](http://www.jbc.org/)

Downloaded from http://www.jbc.org/ on July 25, 2018
Fig. 2. Processing of a PLAP-BP2 fusion protein reflects sterol status of cells. A, proteolytic cleavage and secretion of the PLAP-BP2 fusion protein (after Sakai et al. (25)). Cells were transfected with plasmid encoding a chimeric SREBP-2 protein containing the secreted form of human placental alkaline phosphatase fused to the amino-terminal half of human SREBP-2 (designated pCMV-PLAP-BP2(513–1141)). PLAP-BP2 is cleaved co-translationally by signal peptidase. The PLAP remains membrane-bound unless it is cleaved from the carboxyl half of SREBP-2 by site-1 protease in the Golgi (i.e., when ER cholesterol levels are low), whereupon it is secreted into the culture medium, which is assayed for alkaline phosphatase activity by luminometry. B, sterols and INSIG-1 co-transfection decrease PLAP-BP2 cleavage. C, 25HC alone suppresses PLAP-BP2 cleavage. Triplicate wells of CHO-K1 cells were transfected with pCMV-PLAP-BP2(513–1141) (0.25 μg) and pGFP-SCAP (0.125 μg) and for Panel B, the indicated amount of pCMV-INSIG-1-Myc. The total amount of DNA was adjusted to 1 μg (B) or 0.5 μg (C) with pcDNA3.1 empty vector. Cells were incubated in medium D in the absence or presence of sterols (25HC (1 μM) or/and cholesterol (10 μM)) for 16 h, and medium was assayed for PLAP secretion by luminometry, and cell protein was determined. Values are means ± S.E. (composite of two separate experiments for Panel C). There is no error bar in the sterol-free control in Panel C, because this condition was set at 100 for all three separate experiments.

(7), and [14C]oleoyl-CoA (24 μM, 0.1 μCi). The reaction mixture was preincubated (30 min, 37 °C) in a shaking water bath. The reaction was started by the addition of [14C]oleoyl-CoA and stopped after 10 min by addition of ice-cold ethanol (1 ml). Lipids were extracted, and the extent of cholesterol esterification was determined as described above. ACAT activity was expressed as picomoles of [14C]cholesteryl ester formed per min per mg of microsomal protein.

Analysis of SREBP Processing by Western Blotting—On day 0, CHO-K1 cells were set up at 8 × 10⁵ cells/100-mm dish (in duplicate) in medium B. On day 2, cells were switched to medium D and incubated for 16 h. On day 3, cells were switched to fresh medium D and incubated with various additions (0–1 μg/ml 25HC with or without 10 μM progesterone) for 0–4 h. Cells were harvested, and membrane and nuclear extract fractions were prepared and analyzed by 8% SDS-PAGE and Western blotting as described previously (7, 28).

PLAP-BP2 Cleavage—On day 0, CHO-K1 cells were set up at 1.2 × 10⁶ cells/well in 12-well plate in medium A. On day 1, triplicate wells of cells were refed fresh medium A and then transfected with the indicated plasmids using FuGENE 6 transfection reagent according to the manufacturer's instructions (Roche Diagnostics). A ratio of 1:3 plasmid DNA to FuGENE 6 reagent was used. After incubation for 5–6 h, the cells were rinsed with PBS and incubated with medium D in the absence or presence of test agents as indicated in the figure legends. After incubation for 16 h, the medium was removed and centrifuged (20,800 × g, 20 min, 4 °C). An aliquot of supernatant (50 μl) was diluted, heat-treated (30 min, 65 °C) to inactivate non-placental alkaline phosphatase, and assayed for secreted alkaline phosphatase activity using the SEAP gene reporter assay kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. After 10–20 min, chemiluminescence was quantified on a Wallac Microbeta luminometer. Cells were lysed with 0.1M NaOH (0.25 ml), and proteins were determined using the bicinchoninic acid protein assay kit (Pierce) to normalize secreted alkaline phosphatase activity (expressed as relative light units).

RESULTS

The regulatory pool of cholesterol is located in the ER and is key to how mammalian cells sense and respond to changes in cellular cholesterol levels. The aim of this study was to gain greater insight into how cellular cholesterol levels are regulated via transport of cholesterol to the cholesterol homeostatic machinery in the ER. We have focused on cholesterol transport induced by the oxysterol, 25HC. Although the precise mechanism remains obscure, the current view is that oxysterols like 25HC trigger intracellular cholesterol transport to the ER (29). In support of this contention, others have shown that 25HC activates cholesterol esterification (e.g. (13, 19–21)) and is a potent suppressor of SREBP processing (e.g. Refs. 5–7 and 15). Our results confirm these previous reports. When the plasma membrane of CHO-K1 cells was pulsed with [3H]cholesterol, 25HC stimulated esterification of the labeled cholesterol within an hour (Fig. 1A). 25HC suppressed SREBP processing to the
Fig. 3. Does progesterone treatment of CHO-K1 cells block 25HC suppression of SREBP processing? A, the schematic shows that progesterone blocks the ability of 25HC to stimulate esterification of free cholesterol (FC) to cholesteryl esters (CE) by ACAT and questions whether or not 25HC suppression of the SREBP-SCAP system is also blocked by progesterone. The SCAP-SREBP system includes INSIG proteins that can bind to both SCAP (5) and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (50). B, triplicate 60-mm dishes of CHO-K1 cells were incubated with medium D containing 25HC (0–1 μg/ml) in the absence or presence of progesterone (10 μM) for 4 h. Cells were harvested, and free and esterified [3H]cholesterol was determined. Values are means ± S.E. (representative of two separate experiments). C, CHO-K1 cells were incubated with medium D containing 25HC (0–1 μg/ml) in the absence or presence of progesterone (10 μM) for 16 h. Following pulse labeling with [3H]cholesterol/cyclodextrin (10 min, 4 °C), the cells were harvested for preparation of cell fractions. Aliquots of membranes (25 μg) and nuclear extracts (40 μg) were subjected to 8% SDS-PAGE and Western blotting with IgG-7D4 for SREBP-2 (membranes only) and IgG-R139 for SCAP (membranes only). P and N denote the precursor and nuclear (cleaved) forms of SREBP-2, respectively. Nitrocellulose membranes were exposed to film for 30 s. D, triplicate wells of CHO-K1 cells in a 12-well plate were transfected with pCMV-PLAP-BP2(513–1141) and pGFP-SCAP as described in Fig. 2C. Cells were incubated in medium D containing 25HC (0–1 μg/ml) in the absence or presence of progesterone (10 μM) for 16 h, and medium was assayed for PLAP secretion.

SREBP processing is determined under conditions of sterol deprivation, i.e. in lipoprotein-deficient medium in the presence of a cholesterol synthesis inhibitor (compactin). Therefore, suppression of SREBP processing by 25HC is consistent with the idea that 25HC shifts an existing pool of cellular cholesterol to the cholesteryl esterification and suppression of PLAP-BP2 processing. Like progesterone, nigericin, a potassium ionophore, and monensin, were shown previously to inhibit the ability of 25HC to stimulate cholesterol esterification (35, 36). Previously (21, 33, 34), progesterone was found to inhibit 25HC-induced cholesterol esterification, indicating that progesterone inhibits cholesterol delivery to the ER. The question is whether or not progesterone also inhibits the suppressive effect of 25HC on SREBP processing (Fig. 3A). If progesterone were to inhibit the expansion of 25HC of the ER regulatory pool of cholesterol, it would also be predicted to relieve 25HC-mediated suppression of SREBP-2 processing, causing increased nuclear SREBP-2. In accordance with previous reports (21, 33, 34), we found that progesterone inhibited 25HC-induced cholesterol esterification (Fig. 3B). However, contrary to expectations, progesterone treatment tended to decrease the nuclear form of SREBP-2 (Fig. 3C). Moreover, there was no indication that the response of SREBP processing to 25HC was blunted (Fig. 3C). This result was confirmed using the PLAP-BP2 reporter assay, i.e. 25HC still inhibited PLAP-BP2 processing in the presence of progesterone (Fig. 3D). In further experiments, varying concentrations of progesterone and two other agents (nigericin and monensin) were tested for their ability to block the effect of 25HC on cholesterol esterification and suppression of PLAP-BP2 processing. Like progesterone, nigericin, a potassium ionophore, and monensin, a sodium ionophore, were shown previously to inhibit the ability of 25HC to stimulate cholesterol esterification (35, 36). Results in Fig. 4 are presented relative to the effect induced by 1 μg/ml 25HC, so that 0% signifies that this effect was completely blocked, whereas 100% signifies that the effect was unaltered. In agreement with previous studies (21, 33–36), all
three agents decreased the ability of 25HC to stimulate cholesterol esterification in a dose-dependent fashion (Fig. 4, A, C, and E). Importantly, these agents did not inhibit the ability of 25HC to suppress PLAP-BP2 processing (Fig. 4, B, D, and F), indicating that cholesterol esterification does not necessarily reflect cholesterol transport to the regulatory pool in the ER.

To validate our approach using pharmacologic agents, we performed positive control experiments. To date, the hypcholesterolemic agent, LY295427, is the only compound shown to block the effect of 25HC both in increasing cholesterol esterification and suppressing SREBP processing (37). LY295427 is a 3α-hydroxysteroid (4α-allylcholestan-3α-ol) and was identified for its ability to activate the LDL-receptor promoter in the presence of 25HC (38). Its 3β-isomer (LY306039) is inactive (37) and was used as a control. We found that LY295427 (but not LY306039) inhibited 25HC-induced effects on cholesterol esterification at relatively high concentrations (Fig. 5A). In agreement with previous work (30, 37), we found that the active isomer, LY295427, also inhibited the effect of 25HC of suppressing SREBP (PLAP-BP2) processing and that this effect was maximal at 20 μM (Fig. 5B). Therefore, our experiments with LY295427 confirm previous reports, and indicate the soundness of our pharmacologic approach to dissect the effects of 25HC on cholesterol esterification and SREBP processing.

A trivial explanation of why a pharmacologic agent could have a different effect on cholesterol esterification by ACAT and on the cholesterol homeostatic machinery would be that the agent inhibits ACAT activity, and therefore may not directly influence cholesterol transport to the ER. Others have reported that nigericin and monensin do not inhibit ACAT activity (39). However, under some (40, 41) but not all circumstances (31, 39), progesterone can inhibit ACAT activity. Therefore, we determined ACAT activity in membrane microsomes prepared from CHO-K1 cells treated with 10 μM progesterone for 4 h. We found no effect of progesterone (mean ± S.E.: 454 ± 17 versus 430 ± 22 pmol/min/mg of protein for ethanol control versus progesterone, respectively). This in vitro assay was performed under conditions of cholesterol excess, meaning that substrate delivery to the enzyme was not limiting. Therefore, the ability of progesterone to block 25HC stimulation of cholesterol esterification in intact cells is not due to a direct inhibitory effect on ACAT activation, supporting the view that progesterone inhibits intracellular cholesterol transport.

ACAT is proposed to maintain the regulatory pool of ER cholesterol through esterification. Inhibition of ACAT might then be predicted to expand the regulatory cholesterol pool and hence to suppress SREBP processing. Under conditions employed in the PLAP-BP2 cleavage assay, 25HC induces cholesterol transport to the ER from an existing cellular pool, because the assay is performed in lipoprotein-deficient serum with cholesterol esterification measured as the relative effect induced by 25HC (1 μg/ml). Cholesterol esterification (A) and PLAP-BP2 cleavage (B) were determined as outlined in Figs. 3A and 2C, respectively, for the indicated concentrations of LY295427 (solid diamonds) or its inactive isomer, LY306039 (empty circles).

**FIG. 4.** Effect of 25HC on cholesterol esterification and PLAP-BP2 cleavage are dissociable in CHO-K1 cells. Results are presented as the relative effect induced by 25HC (1 μg/ml) so that 0% signifies that this effect is completely blocked, whereas 100% signifies that the effect was unaltered. Cholesterol esterification and PLAP-BP2 cleavage were determined as outlined in Figs. 3A and 2C, respectively.

**Fig. 5.** LY295427 inhibits the 25HC-induced effect on both cholesterol esterification and PLAP-BP2 cleavage in CHO-K1 cells. A and B, results are presented as the relative effect induced by 25HC (1 μg/ml). Cholesterol esterification (A) and PLAP-BP2 cleavage (B) were determined as outlined in Figs. 3A and 2C, respectively, for the indicated concentrations of LY295427 (solid diamonds) or its inactive isomer, LY306039 (empty circles).

**DISCUSSION**

Mechanisms by which cholesterol is transported to the ER remain largely undefined. By questioning our current knowledge based on cholesterol esterification, we have made an initial and necessary step toward re-evaluating cholesterol transport to the regulatory pool in the ER. We present two lines of
The authors concluded that several membrane-trafficking pathways may feed ACAT with cholesterol (42).

Another possibility is that there may be more than one pool of ACAT. Subcellular fractionation studies of rat liver concluded that ACAT mostly resides in the rough ER (44, 45). Similarly, Chang et al. (46) showed, by indirect immunofluorescence microscopy, that ACAT in melanoma cells was distributed in a typical ER-like pattern. However, more recently, Khelef et al. (47, 48) observed in murine macrophages, that a significant fraction of ACAT was in a distinct undefined sub-compartment of the ER, close to the trans-Golgi network and endocytic recycling compartment. Further work is required to determine if the dissociation between the effect of 25HC on cholesterol esterification and SREBP processing represents different cholesterol trafficking pathways and/or trafficking to a distinct pool of ACAT.

How the ER gains access to the regulatory pool of cholesterol is an important unresolved issue in our understanding of cholesterol homeostasis. The recent finding that the ER is the site of cholesterol-induced cytotoxicity in macrophages (49) further reinforces the importance of increasing our understanding of cholesterol traffic to and from the ER. In light of our results, we question the security of previous work that inferred cholesterol transport to the ER regulatory pool based solely on cholesterol esterification. We suggest that our current understanding of cholesterol transport to the ER, largely based on such studies, may need reassessing.

**Acknowledgments**—We thank Drs. Michael S. Brown and Joseph L. Goldstein for generously providing reagents. Also thanks to Drs. Ingrid Gelissen and Malcolm Lyons for critically reading this manuscript. LY295427 and LY306039 were kindly donated by Eli Lilly.

**REFERENCES**

1. Brown, M. S., and Goldstein, J. L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 11041–11048
2. Steck, T. L., and Lange, Y. (2002) *Dev. Cell* **3**, 306–308
3. Anderson, R. G. (2003) *Trends Cell Biol.* **13**, 534–539
4. Rawson, R. B. (2003) *Nat. Rev. Mol. Cell. Biol.* **4**, 631–640
5. Yang, T., Espenshade, P. J., Wright, M. E., Yabe, D., Gong, Y., Aebersold, R., Goldstein, J. L., and Brown, M. S. (2002) *Cell* **110**, 489–500
6. Yabe, D., Brown, M. S., and Goldstein, J. L. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 12753–12758
7. Brown, A. J., Sun, L., Faramisco, J. D., Brown, M. S., and Goldstein, J. L. (2002) *Mol. Cell* **10**, 237–245
8. Adams, C. M., Goldstein, J. L., and Brown, M. S. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 10647–10652
9. Espenshade, P. J., Li, W. F., and Yabe, D. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 11694–11699
10. Lange, Y., Ye, J., and Steck, T. L. (2002) *Biochem. Biophys. Res. Commun.* **290**, 488–493
11. Simons, K., and Ikonen, E. (2000) *Science* **290**, 1721–1726
12. Scheidt, H. A., Muller, P., Herrmann, A., and Huster, D. (2003) *J. Biol. Chem.* **278**, 45563–45569
13. Lange, Y., and Steck, T. L. (1997) *J. Biol. Chem.* **272**, 13103–13108
14. Debruy, P., Nash, E. A., Nekiason, D. W., and Metherhal, J. R. (1997) *J. Biol. Chem.* **272**, 1026–1031
15. Wang, X., Sato, R., Brown, M. S., Hua, X., and Goldstein, J. L. (1994) *Cell* **77**, 53–62
16. Rowe, A. H., Argmann, C. A., Edwards, J. Y., Sawyez, C. G., Morand, O. H., Hegele, R. A., and Huff, M. W. (2003) *Circ. Res.* **93**, 717–725
17. Land, E. G., Kerr, T. A., Sakai, J., Li, W. P., and Russell, D. W. (1998) *J. Biol. Chem.* **273**, 34316–34327
18. Hall, E., Hylemon, P., Vlahcevic, Z., Mallonee, D., Valerie, K., Avadhani, N., and Pandak, W. (2001) *Ann. J. Physiol.* **281**, 4295–4301
19. Brown, M. S., Dana, S. E., and Goldstein, J. L. (1975) *J. Biol. Chem.* **250**, 4925–4927
20. Field, F. J., and Mathur, S. N. (1983) *J. Lipid Res.* **24**, 1049–1059
21. Müller, S. C., and Melyanoych, G. (1984) *J. Lipid Res.* **25**, 991–999
22. Goldstein, J. L., Basu, S. K., and Brown, M. S. (1983) *Methods Enzymol.* **98**, 241–260
23. Sakai, J., Nothuhrf, A., Cheng, D., Ho, Y. K., Brown, M. S., and Goldstein, J. L. (1997) *J. Biol. Chem.* **272**, 20213–20221
24. Yang, J., Sato, R., Goldstein, J. L., and Brown, M. S. (1994) *Genes Dev.* **8**, 1910–1919
25. Sakai, J., Rawson, R. B., Espenshade, P. J., Cheng, D., Seegmiller, A. C., Goldstein, J. L., and Brown, M. S. (1998) *Mol. Cell* **2**, 505–514
26. Nothuhrf, A., Yabe, D., Goldstein, J. L., Brown, M. S., and Espenshade, P. J. (2000) *Cell* **102**, 315–323
27. Billheimer, J. T., Tavani, D., and Nes, W. R. (1981) *Anal. Biochem.* **111**, 331–335
28. Dellose-Boyd, R. A., Brown, M. S., Li, W. P., Nothuhrf, A., Goldstein, J. L., and...
29. Socci, R. E., and Breslow, J. L. (2004) *Arterioscler. Thromb. Vasc. Biol.*, **24**, 1150–1160
30. Janowski, B. A. (2002) *Proc. Natl. Acad. Sci. U. S. A.*, **99**, 12675–12680
31. Lange, Y. (1994) *J. Biol. Chem.*, **269**, 3411–3414
32. Wiegand, V., Chang, T. Y., Strauss, J. F., 3rd, Fahrenholz, F., and Gimpl, G. (2003) *FASEB J.*, **17**, 782–784
33. Zhang, J., Ming, L. J., Sjovall, J., Cook, H. W., Ridgway, N. D., and Byers, D. M. (1999) *J. Steroid. Biochem. Mol. Biol.*, **70**, 123–131
34. Casinato, F., and Bruni, A. (2002) *Lipids*, **37**, 53–59
35. Lange, Y., and Steck, T. L. (1994) *J. Biol. Chem.*, **269**, 29371–29374
36. Underwood, K. W., Jacobs, N. L., Howley, A., and Liscum, L. (1998) *J. Biol. Chem.*, **273**, 4266–4274
37. Janowski, B. A., Shan, B., and Russell, D. W. (2001) *J. Biol. Chem.*, **276**, 45408–45416
38. Lin, H. S., Rampersaud, A. A., Archer, R. A., Pawlak, J. M., Beavers, L. S., Schmidt, R. J., Kauffman, R. P., Bensch, W. R., Bumol, T. F., and Apelgren, L. D., Encho, P. I., Perry, D. N., McClure, D. B., and Gadek, R. A. (1995) *J. Med. Chem.*, **38**, 277–288
39. Lange, Y., Ye, J., and Strebel, F. (1995) *J. Lipid Res.*, **36**, 1092–1097
40. Goldstein, J. L., Faust, J. R., Dygos, J. H., Chorvat, R. J., and Brown, M. S. (1978) *Proc. Natl. Acad. Sci. U. S. A.*, **75**, 1877–1881
41. Mazzone, T., Krishna, M., and Lange, Y. (1995) *J. Lipid Res.*, **36**, 544–551
42. Hota-Vuori, M., Tanhuonpaa, K., Mobius, W., Somerharju, P., and Ikonen, E. (2002) *Mol. Biol. Cell*, **13**, 3107–3122
43. Liscum, L., Ruggiero, R. M., and Faust, J. R. (1989) *J. Cell Biol.*, **108**, 1625–1636
44. Balasubramaniam, S., Venkatesan, S., Mitropoulos, K. A., and Peters, T. J. (1978) *Biochem. J.*, **174**, 863–872
45. Hashimoto, S., and Fogelman, A. M. (1980) *J. Biol. Chem.*, **255**, 8678–8684
46. Chang, C. C., Chen, J., Thomas, M. A., Cheng, D., Del Priore, V. A., Newton, R. S., Pape, M. E., and Chang, T. Y. (1995) *J. Biol. Chem.*, **270**, 29532–29540
47. Khelef, N., Buton, X., Beatini, N., Wang, H., Meiner, V., Chang, T. Y., Farese, R. V., Jr., Maxfield, F. R., and Tabas, I. (1998) *J. Biol. Chem.*, **273**, 11218–11224
48. Khelef, N., Soe, T. T., Quehenberger, O., Beatini, N., Tabas, I., and Maxfield, F. R. (2000) *Arterioscler. Thromb. Vasc. Biol.*, **20**, 1769–1776
49. Feng, B., Yao, P. M., Li, Y., Devlin, C. M., Zhang, D., Harding, H. P., Sweeney, M., Rong, J. X., Kuriakose, G., Fisher, E. A., Marks, A. R., Ron, D., and Tabas, I. (2003) *Nat. Cell Biol.*, **5**, 781–792
50. Sever, N., Yang, T., Brown, M. S., Goldstein, J. L., and DeBose-Boyd, R. A. (2003) *Mol. Cell*, **11**, 25–33
Effects of 25-Hydroxycholesterol on Cholesterol Esterification and Sterol Regulatory Element-binding Protein Processing Are Dissociable: IMPLICATIONS FOR CHOLESTEROL MOVEMENT TO THE REGULATORY POOL IN THE ENDOPLASMIC RETICULUM

Ximing Du, Yen H. Pham and Andrew J. Brown

J. Biol. Chem. 2004, 279:47010-47016.
doi: 10.1074/jbc.M408690200 originally published online August 17, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408690200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 31 of which can be accessed free at
http://www.jbc.org/content/279/45/47010.full.html#ref-list-1