The Niemann-Pick type C1 (NPC1) protein is a key participant in intracellular trafficking of low density lipoprotein cholesterol, but its role in regulation of sterol homeostasis is not well understood. To characterize further the function of NPC1, we generated stable Chinese hamster ovary (CHO) cell lines overexpressing the human NPC1 protein (CHO/NPC1). NPC1 overexpression increases the rate of trafficking of low density lipoprotein cholesterol to the endoplasmic reticulum and the rate of delivery of endosomal cholesterol to the plasma membrane (PM). CHO/NPC1 cells exhibit a 1.5-fold increase in total cellular cholesterol and up to a 2.9-fold increase in PM cholesterol. This increase in PM cholesterol is closely paralleled by a 3-fold increase in de novo cholesterol synthesis. Inhibition of cholesterol synthesis results in marked redistribution of PM cholesterol to intracellular sites, suggesting an unsuspected role for NPC1 in internalization of PM cholesterol. Despite elevated total cellular cholesterol, CHO/NPC1 cells exhibit increased cholesterol synthesis, which may be attributable to both resistance to oxysterol suppression of sterol-regulated gene expression and to reduced endoplasmic reticulum cholesterol levels under basal conditions. Taken together, these studies provide important new insights into the role of NPC1 in the determination of the levels and distribution of cellular cholesterol.
NPC Overexpression in CHO Cells

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium, Ham’s F-12 medium, fetal calf serum, glutamine, penicillin/streptomycin, and Lipofectamine Plus were obtained from Life Technologies, Inc. Lipoprotein-deficient fetal calf serum was obtained from Cocalico Labs. Cholesterol oxidase was obtained from Calbiochem. U18666A was obtained from Biomol. Compak resin, β-hydroxysteroylproteolyzer oxidin (C5), filipin, compactin, 3α-[3H]cholesterol, LDL, mevalonic acid, cholesterol-oxysterolin, and lysozyme were obtained from Sigma. LysoSensor Green DND-153 was obtained from Invitrogen. Oleic acid, triolein, and cholesteryl oleate were obtained from Molecular Probes. [3H]cholesterol and [14C]acetate were obtained from PerkinElmer Life Sciences.

Plasmids—The human NPC1 cDNA in pSPORT-NPC1 was provided by J. Strauss (University of Pennsylvania). A ΔU3hNPC1 construct was created by polymerase chain reaction using pSPORT-NPC1 as template and the following primers: 5’-GCTGTAGCTGCCGCACTGCGTGCGCCGC-3’ and 5’-CCGGATCCAGGATGCCTCGGAGGAGGCG-3’. The 3.8-kilobase polymerase chain reaction product was digested with XhoI and Bsm BI and subcloned into the XhoI and Bsm BI cloning sites of the ΔU3 retroviral construct (12). All polymerase chain reaction-derived sequences were confirmed by ABI Prism automated sequencing. The SYN sterol regulatory element (SRE) plasmid was a gift of R. E. Millard, K. Srivastava, L. M. Traub, J. E. Schaffer, and D. S. Ory.

Cell Lines—CHO-K1 cells were obtained from ATCC (CRL-9618). CT60 cells, a CHO cell line that harbors mutations in NPC1 and SCAP, were provided by T. Y. Chang (Dartmouth College). M12 cells are mutant CHO-K1 cells that contain a deletion of the NPC1 locus. To generate the CHO/NPC1 cell lines, CHO-K1 cells were infected with retrovirus prepared by transient transfection of 293GPG-packaging cells with the ΔU3hNPC1 construct (12). The retrovirally infected cells were plated at limiting dilution, and colonies were screened by Western blot analysis of microsomal fractions for human NPC1 expression (described under “Western Blot Analysis”).

Cell Culture and Preparation of Reconstituted LDL Cells—CHO-NPC1 cells were maintained in monolayer culture at 37°C with 5% CO2. All CHO cell lines were maintained in medium A (1:1 Dulbecco’s modified Eagle’s medium:Ham’s F-12, 5% fetal calf serum, 2 mM glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin). Medium B consists of medium A plus 20 μg/ml compactin and 0.5 mM mevalonate. Compak resin and mevalonate were described above. CHO-GPG cells were generated as described by Mendez et al. Dulbecco’s modified Eagle’s medium with 10% fetal calf serum, 2 mM glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 2 μg/ml puromycin, 0.3 μg/ml G418, and 1 μg/ml tetracycline. LDL labeled with [3H]cholesterol and linoleate (CL) ([3H]LDL) was prepared as described above with the CH2Cl2:methanol (1:1) and the cells (hexane/isopropyl alcohol (2:3), a recovery standard was added (0.0005 μCi [3H]cholesterol oleate for media lipid samples; 80 μg cholesterol, 30 μg cholesterol oleate, and 0.0005 μCi [3H]cholesterol oleate for cellular lipid samples), and lipids were dried under nitrogen. Lipids were extracted from media by CH2Cl2:methanol (2:1), and cellular lipids were separated by TLC as described above using heptane:ether:acetic acid (90:30:1) as the solvent. [3H]cholesterol was quantified by scintillation counting. Protein determinations were performed using the MicroBCA assay (Pierce). LDL-specific cholesterol esterification was determined by subtracting esterification rates for non-LDL-fed cells from LDL-fed cells.

Cholesterol Efflux Assay—On day 0, CHO, M12, and CHO/NPC1 cells were seeded in triplicate (5 × 103 cells/35-mm well) in medium A. On day 2, the cells were washed twice with PBS and refed medium B. On day 3, the cells were fed 20 μg/ml of [3H]CL-LDL in medium B plus 20 μg/ml progesterone. On day 4, the cells were washed three times with PBS and incubated with medium B plus 2% CD for up to 2 h. Lipids were extracted from the media (CH2Cl2:methanol (2:1)) and the cells (hexane/isopropyl alcohol (2:3), a recovery standard was added (0.0005 μCi [3H]cholesterol oleate for media lipid samples; 80 μg cholesterol, 30 μg cholesterol oleate, and 0.0005 μCi [3H]cholesterol oleate for cellular lipid samples), and lipids were dried under nitrogen. Lipids were extracted from media by CH2Cl2:methanol (2:1), and cellular lipids were separated by TLC as described above using heptane:isopropyl alcohol (2:3). The [3H]cholesterol efflux was determined as the amount of [3H]cholesterol in the medium divided by the sum of the [3H]cholesterol in medium plus the [3H]cholesterol in the cell extract.

Cholesterol Oxidase Treatment—Cholesterol oxidase treatment was performed as described previously (9). On day 0, CHO, M12, and CHO/NPC1 cells were seeded in triplicate (5 × 103 cells/35-mm well) in medium A. On day 2, the cells were washed twice with PBS and refed medium B. On day 3, the cells were fed either medium B or medium C with 1 μCi [3H]cholesterol/well. On day 4, each well was washed three times with Tris-buffered saline plus 2 mg/ml bovine serum albumin (Sigma) at 4°C for 5 min on a shaker, and rapidly washed twice with PBS at room temperature. The cells were fixed in 1% glutaraldehyde in PBS for 10 min at room temperature and incubated with cholesterol oxidase (2 units/well) and sphingomyelinase (0.1 units/well) in Ham’s F-12 for 30 min at 37°C. The cells were washed twice with PBS, and lipids were extracted as described above. A chromatography recovery standard was added (20 μg cholesterol, 40 μg cholestanone, 30 μg cholesterol oleate, 0.0005 μCi [3H]cholesterol oleate), and the samples were dried under nitrogen. TLC was performed as above using heptane:ethyl ether:acetic acid (90:40:4) as the solvent. [3H]cholesterol was quantified by scintillation counting. Protein determinations were performed as described above using the MicroBCA assay. The percent cholesterol efflux was determined as the amount of [3H]cholesterol in the medium divided by the sum of the [3H]cholesterol in medium plus the [3H]cholesterol in the cell extract.

Assay of de Novo Cholesterol Synthesis—Metabolic labeling of de novo synthesized cholesterol was performed as described previously (18). On day 0, CHO, M12, CT60, and CHO/NPC1 cells were seeded in triplicate (2 × 104 cells/35-mm well) in medium A. On day 1, the cells were washed three times with PBS and refed medium B. On day 2, the cells were washed three times with PBS and refed medium B with 0.5 μCi [3H]acetate (25 μCi/mmol). After a 2-h incubation, the cells were washed three times with Tris-buffered saline at 4°C, and lipids were extracted as described above. A chromatography recovery standard...
and was added (20 μg cholesterol, 30 μg cholesteryl oleate, 0.002 μCi [3H]cholesterol), and the samples were dried under a stream of nitrogen. TLC and [3H]cholesterol quantification was performed as described under “Cholesterol Oxidase Treatment,” and protein determination was performed using the MicroBCA assay.

Luciferase Reporter Assay—On day 0, CHO, CT60, and CHO/NPC1 cells were plated in duplicate (6 × 10⁴ cells/60-mm dish) in medium A. On day 1, the cells were co-transfected with 1.5 μg of pSyn SRE and 0.5 μg of pCMVβgal. Four hours post-transfection, the media was changed to medium C supplemented with 0–1.0 μg/ml 25-HC. On day 2, cells were harvested in reporter lysis buffer (Promega), and luciferase and β-galactosidase assays were performed in duplicate for each sample. The luciferase activity in the transfected cells was normalized to β-galactosidase expression to correct for transfection efficiency.

In Vitro Cholesterol Esterification Assay—The in vitro esterification assay was performed as described by Lange and Steck (19). Cells were trypsinized, pelleted, and washed in 0.25M sucrose, 5 mM sodium phosphate, pH 7.5, and swelled on ice for 10 min. The cells were homogenized with a Dounce homogenizer (100–200 strokes), centrifuged to remove large particles, and adjusted to 1 mM dithiothreitol and 1 mg/ml bovine serum albumin. The esterification reaction was started by the addition of 25 μM [14C]oleoyl-CoA followed by incubation for 2 h at 37 °C. After extraction with CH₃Cl:methanol (2:1) and addition of a recovery standard (40 μg cholesterol, 30 μg cholesteryl oleate, 0.002 μCi [3H]cholesterol), lipids were dried under nitrogen. Cholesteryl oleate was recovered by TLC and quantified as described under “LDL-stimulated Cholesterol Esterification Assay.” Protein determinations were performed using the BCA assay.

RESULTS

Isolation of CHO Cell Lines Overexpressing NPC1—To study the function of NPC1, we used a retroviral expression system to establish stable CHO cell lines that overexpress NPC1. The human NPC1 cDNA was cloned into the ΔU3 retroviral vector and transfected into 293GPG-packaging cells to generate high titer virus encoding NPC1 (12). The virus was used to infect and transfected into 293GPG-packaging cells to generate high titer virus encoding NPC1 (12). The virus was used to infect CHO cells, CT60, and CHO/NPC1 and human normal skin fibroblasts (NSF) 80 °C, 10–1274 (data not shown) (20). The 220- and 170-kDa bands likely represent heterogeneously glycosylated NPC1 and have been observed by others (21). Because the COOH termini of human and hamster NPC1 only share 78% identity from residues 1261–1278, the NPC1 antibody demonstrates a preference for recognition of human over endogenous CHO sequences (note the 8-fold difference in amount of protein loaded in CHO versus CHO/NPC1 and human normal skin fibroblasts (NSF) lanes). Therefore, to estimate levels of transgene expression, we compared NPC1 expression in the CHO/NPC1 cell lines with the endogenous NPC1 expression in human NSF. Among the CHO/NPC1 cell lines, expression of NPC1 varies over a 12-fold range, with NPC1-1 ~ NPC1-27 > NPC1-9 > NPC1-28. The lowest expressing CHO/NPC1 cell line, NPC1-28, expressed human NPC1 at a level 1.3-fold above NSF. The highest expressing CHO/NPC1 cell line, NPC1-1, expressed human NPC1 at a level 15-fold above NSF.

Immunofluorescence studies were performed to confirm the expression of NPC1 in these cell lines and to establish that when overexpressed in CHO cells, the human NPC1 protein distributes to appropriate cellular compartments. We co-stained fixed, permeabilized cells with affinity-purified antibody to the NPC1 COOH terminus and with filipin, a fluorescent polyene antibiotic that specifically binds unesterified cholesterol (22). In wild-type CHO cells under basal conditions (Fig. 2A), weak staining for NPC1 was observed in granular structures that have been identified in previous studies as late endosomes (9). This staining was not observed in controls with secondary antibody-staining alone (data not shown). Filipin staining was observed in a Golgi-like pattern and at the cell periphery (Fig. 2B). NPC1-staining was not observed in the NPC1-null, filipin-positive M12 cell line (Fig. 2C and D). In CHO/NPC1 cell lines, robust NPC1 staining was observed in granular and reticular structures (Fig. 2E). Filipin primarily stained the PM and granular structures in the CHO/NPC1 cells (Fig. 2F). The intensity of filipin staining exceeded that of parental CHO cells, suggesting that these cells have increased cellular cholesterol content.

To show that overexpressed NPC1 is also appropriately localized in the setting of pharmacologic block of cholesterol...
TRAFFICKING, CHO/NPC1 cells were incubated with U18666A. This hydrophobic amine inhibits mobilization of lysosomal cholesterol and results in the NPC mutant phenotype (10). U18666A-treated cells were co-stained with an antibody for NPC1, filipin, and a LysoSensor probe that specifically stains acidic organelles (23). After treatment with U18666A, there was a decrease in the granular NPC1-staining pattern and appearance of multiple, large, perinuclear vesicular structures whose periphery stains intensely for NPC1 (Fig. 3A, see arrows). Many of these structures are cholesterol-rich as demonstrated by filipin staining (Fig. 3B) and co-stain with LysoSensor probe (Fig. 3C), consistent with a late endosomal or lysosomal localization. This pattern of circumferential staining around cholesterol-containing vesicular structures after U18666A treatment is similar to the staining we have observed in mutant cell lines that have normal NPC1 expression but genetic blocks in the cholesterol-trafficking pathway.3

**Overexpressed NPC1 Affects Cellular Cholesterol Trafficking**—To characterize the effects of NPC1 overexpression on LDL cholesterol trafficking, we compared LDL-stimulated cholesterol esterification in wild-type CHO, M12, and CHO/NPC1 cell lines. In normal cells, uptake of LDL cholesterol expands the cellular cholesterol pool and activates acyl-CoA:cholesterol O-acyltransferase (ACAT), catalyzing the esterification of both de novo-synthesized and LDL-derived cholesterol in the ER (3). Cells were incubated in medium B, fed LDL 50 μg/ml overnight, and pulsed for 2 h with [3H]oleate. Esterification rates, as determined by incorporation of [3H]oleate into [3H]cholesterol, were determined by subtracting esterification rates for non-LDL-fed wells from LDL-fed wells. Esterification rates (pmol/min/mg) were normalized to wild-type CHO cells. Values are means ± S.E. and are representative of four independent experiments.

**Increased delivery of cholesterol to the PM in CHO/NPC1 cells**—To characterize the effects of NPC1 overexpression on LDL cholesterol trafficking, we compared LDL-stimulated cholesterol esterification in wild-type CHO, M12, and CHO/NPC1 cell lines. In normal cells, uptake of LDL cholesterol expands the cellular cholesterol pool and activates acyl-CoA:cholesterol O-acyltransferase (ACAT), catalyzing the esterification of both de novo-synthesized and LDL-derived cholesterol in the ER (3). Cells were incubated in medium B, fed LDL 50 μg/ml overnight, and pulsed for 2 h with [3H]oleate. Esterification rates, as determined by incorporation of [3H]oleate into [3H]cholesterol, were determined by subtracting esterification rates for non-LDL-fed wells from LDL-fed wells. Esterification rates (pmol/min/mg) were normalized to wild-type CHO cells. Values are means ± S.E. and are representative of four independent experiments.

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**NPC1 Overexpression in CHO Cells**

NPC1 cells were incubated with progesterone and [3H]CL-LDL for 24 h and treated with 2% CD for up to 2 h, and the rates of cholesterol efflux were measured during a progesterone wash-out phase. After a 2-h incubation with CD, cholesterol efflux from the M12 cells was decreased by 30% as compared with wild-type CHO cells (Fig. 5). NPC1 overexpression increased cholesterol efflux in the NPC1–27 and NPC1–28 cell lines by 43 and 21%, respectively, as compared with wild-type CHO cells. These findings are consistent with previous studies that support a role for NPC1 in the mobilization of lysosomal cholesterol (2, 10, 11). Taken together, the immunofluorescence, cholesterol esterification, and cholesterol efflux studies provide evidence that the exogenously expressed NPC1 in our stable cell lines is targeted to appropriate cellular membranes and functions in the trafficking of LDL cholesterol in a biologically relevant manner.

NPC1 Overexpression Affects Cholesterol Homeostasis—We anticipated that increased trafficking of LDL cholesterol to the ER and PM in the CHO/NPC1 cells might alter the partitioning of cellular cholesterol between the PM and interior membrane compartments and/or affect the size of cellular cholesterol pools. To study the potential effect of NPC1 overexpression on cellular distribution of cholesterol, cells were labeled with [3H]cholesterol and treated with cholesterol oxidase, which oxidizes cholesterol to cholestenone. Quantification of [3H]cholesterol (cholesterol in the oxidase-accessible pool) and unesterified [3H]cholesterol provides a measure of PM and intracellular cholesterol pools, respectively (25). We performed these studies under conditions of lipoprotein starvation in the presence and in the absence of compactin, an inhibitor of de novo cholesterol synthesis. As compared with wild-type CHO cells, M12 cells grown in the absence of compactin demonstrated a modest reduction (26% decrease) in the cholesterol content of the oxidase-accessible (PM) pool (Fig. 6, black bars). This finding is consistent with the defect in M12 cells in mobilization of endosomal cholesterol to the PM. In contrast, NPC1-overexpressing cell lines demonstrated a dose-dependent increase in PM cholesterol content (up to 2.9-fold) in the presence of compactin. Strikingly, growth of CHO/NPC1 cells in the presence of compactin (Fig. 6, gray bars) reduced PM cholesterol to levels below that of wild-type CHO cells, whereas intracellular cholesterol levels were increased up to 2.5-fold. As a percentage of the values for wild-type CHO cells, total cellular cholesterol in CHO/NPC1 cells was increased (up to 1.5-fold) and did not significantly differ in the presence or absence of compactin. Taken together, these findings suggest that the increase in PM cholesterol (in the absence of compactin) in the CHO/NPC1 cell lines is due primarily to de novo cholesterol synthesis. Moreover, the redistribution of cholesterol from the PM to the cell interior in the setting of inhibition of de novo cholesterol synthesis implies a role for NPC1 in internalization of PM cholesterol.

To examine the mechanism of the excess sterol accumulation in the PM of CHO/NPC1 cells, we measured rates of de novo cholesterol biosynthesis. CHO, M12, and CHO/NPC1 cell lines were incubated in medium B or medium C, and the cells were pulsed with [3H]cholesterol (1 μCi/well). The cells were fixed and treated with cholesterol oxidase and sphingomyelinase, and lipids were extracted and analyzed by TLC. Incorporation of [3H]cholesterol into the PM cholesterol (A), intracellular cholesterol (B), and total cellular cholesterol (C) pools were determined in the absence (black bars) and presence of compactin (gray bars). Values are means ± S.E. and are representative of two independent experiments. The rate of de novo synthesis (301% of CHO for NPC1–1, 108% of CHO for NPC1–28) closely paralleled the increase in PM cholesterol levels (286% of CHO for NPC1–1, 105% of CHO for NPC1–28).

To determine whether up-regulation of de novo cholesterol synthesis was mediated by altered sterol homeostatic mechanisms involving SREBP’s, we compared the ability of 25-HC to suppress SRE-dependent gene transcription in CHO and CHO/NPC1 cells. CT60 cells were included as a control, since they are known to be sterol-resistant (26, 27). Cells were transfected with pCMV/gal and the pSyn SRE vector, which contains three SREs from the 3-hydroxy-3-methylglutaryl-coenzyme A synthase gene linked to a luciferase reporter. SRE-containing reporter constructs serve as an indicator of the status of SREBP maturation (13, 18). The luciferase activity in the transfected cells was normalized to β-galactosidase expression to correct
for transfection efficiency. As expected, CHO cells responded to incubation with 25-HC by suppression of SRE-dependent transcription at 0.25 μg/ml 25-HC, whereas CT60 cells were resistant to suppression (Fig. 8). CHO/NPC1 cells also failed to suppress to wild-type levels. Resistance to suppression in these cells increased in a dose-dependent manner with increasing oxysterol concentration, whereas resistance in CT60 cells diminished with increasing oxysterol concentration. In four independent experiments, the degree of resistance correlated with the level of NPC1 expression (NPC1–1 > NPC1–9 > NPC1–27). At the highest 25-HC concentration (1 μg/ml), CHO/NPC1 cells were more resistant than CT60 cells.

ER cholesterol levels are thought to play a central role in cholesterol homeostasis through regulation of SCAP/SREBP function (4). To determine whether the perturbations of SCAP/SREBP function in CHO/NPC1 cells resulted from alteration of SREBP function (4). To determine whether the perturbations of SCAP/SREBP function in CHO/NPC1 cells resulted from alteration of CHOSREBP function in CHO/NPC1 cells resulted from alteration of CHONPC1 overexpression inhibits sterol suppression of pSyn SRE expression. On day 0, CHO, CT60, and CHO/NPC1 cells were plated in duplicate (6 × 10^4 cells/60-mm dish) in medium A. On day 1, the cells were co-transfected with 1.5 μg of pSyn SRE and 0.5 μg of pCMVβ-gal. Four hours post-transfection, the media was changed to medium C supplemented with 0–1.0 μg/ml 25-HC. On day 2, cells were harvested, and luciferase and β-galactosidase assays were performed in duplicate. Luciferase activity is normalized for transfection efficiency. As expected, CHO cells responded to incubation with 25-HC by suppression of SRE-dependent transcription at 0.25 μg/ml 25-HC, whereas CT60 cells were resistant to suppression (Fig. 8). CHO/NPC1 cells also failed to suppress to wild-type levels. Resistance to suppression in these cells increased in a dose-dependent manner with increasing oxysterol concentration, whereas resistance in CT60 cells diminished with increasing oxysterol concentration. In four independent experiments, the degree of resistance correlated with the level of NPC1 expression (NPC1–1 > NPC1–9 > NPC1–27). At the highest 25-HC concentration (1 μg/ml), CHO/NPC1 cells were more resistant than CT60 cells.

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cholesterol O-acyltransferase and the mobilization of endosomal cholesterol to the PM (2, 3, 10, 11). Based on these findings, we conclude that the sterol-related phenotype of the CHO/NPC1 cells is due to overexpression of functional NPC1 protein.

In the present study several observations indicate that cholesterol homeostasis in NPC1-overexpressing cells is perturbed. First, CHO/NPC1 cells demonstrate a marked increase in PM cholesterol and total cellular cholesterol levels. We show that de novo cholesterol synthesis is responsible for the increase in PM cholesterol because inhibition of cholesterol synthesis completely abrogates the increase in PM cholesterol. Moreover, the increased rates of cholesterol synthesis (3-fold) in the CHO/NPC1 cells closely parallel the increase in the PM sterol content (2.9-fold). Second, under conditions of cholesterol starvation, CHO/NPC1 cells exhibit a striking redistribution of cholesterol from the PM to the cell interior. In the absence of de novo cholesterol synthesis, PM cholesterol is reduced to levels below that of wild-type CHO cells, even in the cell line with the lowest level of NPC1 expression (NPC1-28). These findings imply an unsuspected role for NPC1 in trafficking of PM cholesterol to intracellular membranes. Third, ER cholesterol levels in the CHO/NPC1 cells are decreased by 20% as compared with wild-type CHO cells. Although the mechanism underlying this decrease is unclear, it is possible that overexpression of NPC1 may adjust the set-point of the ER cholesterol levels either by stimulating cholesterol trafficking or altering the cholesterol content of specific cellular compartments (28). Fourth, despite appropriate oxysterol stimulation of cholesterol movement to the ER, CHO/NPC1 cells fail to effectively suppress SREBP cleavage.

What is the mechanism by which NPC1 overexpression disrupts normal regulation of sterol homeostasis? The increase in cholesterol synthesis in the CHO/NPC1 cells is inappropriate given their increased total cellular cholesterol. Low basal ER cholesterol levels likely provide a stimulus for SREBP proteolysis and thereby contribute to the increased rate of de novo cholesterol synthesis. However, appropriate 25-HC-stimulated movement of cholesterol to the ER occurs in CHO/NPC1 cells, and this influx of cholesterol fails to suppress SRE-dependent gene expression. It is possible that the ER cholesterol in CHO/NPC1 cells is unavailable for regulation of SREBP proteolysis. Alternatively, NPC1 may directly interfere with SCAP/SREBP function. In a recent study, overexpression of the sterol-sensing domain (SSD) of SCAP prevented suppression by sterols of SCAP/SREBP movement to the Golgi (24). A model has been proposed in which the SCAP/SREBP complex binds to an ER retention protein through an interaction involving the SSD of SCAP. The overexpressed SCAP SSD competes with the SCAP/SREBP complex for binding to the putative retention protein, allowing the complex to move to the Golgi despite the presence of sterols. Similarly, overexpression of the SSD-containing NPC1 may interfere with sterol regulation of SCAP/SREBP movement by competing with SCAP for binding to this putative retention protein. Elucidation of the molecular function of NPC1, in general, and of the SSD, in particular, will shed light on the role of this protein in sterol homeostasis.

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