Localization of Mammalian NAD(P)H Steroid Dehydrogenase-like Protein on Lipid Droplets*

Received for publication, February 10, 2003, and in revised form, June 23, 2003
Published, JBC Papers in Press, July 1, 2003, DOI 10.1074/jbc.M301408200

Masato Ohashi†‡, Noboru Mizushima†, Yukiko Kabeya†, and Tamotsu Yoshimori‡

From the †Department of Molecular Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, and ‡Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, and Department of Cell Genetics, National Institute of Genetics, Mishima 411-8540, Japan

Mammalian enzymes in late cholesterol biosynthesis have been localized uniformly over the endoplasmic reticulum by enzymatic methods. We report here the first mammalian cholesterol biosynthetic enzyme unequivocally localized at the surface of intracellular lipid storage droplets. NAD(P)H steroid dehydrogenase-like protein (Nsdhl), a mammalian C-3 sterol dehydrogenase involved in the conversion of lanosterol into cholesterol, was localized on lipid droplets by immunofluorescence microscopy and subcellular fractionation. Nsdhl was localized on lipid droplets even when cell growth exclusively depended on cholesterol biosynthesis mediated by this enzyme. Depletion of fatty acids in culture medium reduced the development of lipid droplets and caused Nsdhl redistribution to the endoplasmic reticulum. Elevating oleic acid in medium induced well-developed, Nsdhl-positive lipid droplets, and simultaneously caused a reduction in cellular conversion of lanosterol into cholesterol. Manipulated human NSDHL with a missense mutation (G205S) causing a human embryonic developmental disorder, congenital hemidysplasia with ichthyosiform nevus and limb defects (CHILD) syndrome, could no longer be localized on lipid droplets. Although the expression of wild-type NSDHL could restore the defective growth of a CHO cholesterol auxotroph, LEX2 in cholesterol-deficient medium, the expression of Nsdhl(G205S) failed to do so. These results point to functional significance of the localization of Nsdhl on lipid droplets. Functional significance was also suggested by the colocalization of Nsdhl on lipid droplets with TIP47, a cargo selection protein for mannose 6-phosphate receptors from late endosomes to the trans-Golgi network. These results add to the growing notion that the lipid droplet is an organelle endowed with more complex roles in various biological phenomena.

pathway. The little cholesterol content in the ER (1) probably keeps the ER membrane more permeable and makes it easier for membrane proteins to translocate through the ER membrane (2). Indeed, an early step in protein translocation across the ER membrane is reversibly inhibited by cholesterol levels significantly lower than those found in the plasma membrane (3). In contrast, the high cholesterol content in the plasma membrane makes this membrane less permeable and, at the same time, thicker. This probably contributes to the targeting of membrane-spanning proteins with longer transmembrane domains to the plasma membrane rather than to the Golgi (2). A significant amount of cholesterol is also found in the trans-Golgi and in endosomes. Sorting and transport events in membrane traffic connecting these organelles are regulated by cholesterol levels (4–8). Thus, cholesterol levels would also be able to regulate the activities of functional molecules by controlling their localization through membrane traffic. In addition, cholesterol can directly bind to proteins, thereby modulating their activities. For example, cholesterol binds to synaptophysin and is required for the biogenesis of synaptic vesicles (9).

To maintain these organelle-specific functions affected by cholesterol levels, the heterogeneous cholesterol distribution must strictly be regulated. However, the mechanisms by which cholesterol is properly distributed among cellular membranes are still enigmatic. To reveal the mechanisms, it is important to understand the cellular topology of cholesterol biosynthesis and transport. The late pathway of mammalian cholesterol biosynthesis has been localized uniformly over the ER by extensive enzymatic studies in which the distribution of newly synthesized sterols and activities of some enzymes were analyzed in subcellular fractionation (10). However, genes of enzymes in mammalian late cholesterol biosynthesis have only recently been identified, and the precise intracellular localization of their product proteins has yet to be determined (11).

Here we report that mammalian NADP(H) steroid dehydrogenase-like protein (Nsdhl) or NSDHL in humans involved in the complex series of reactions that result in the sequential removal of the two C-4 methyl groups from the cholesterol precursor lanosterol is primarily localized to the surface of lipid storage droplets (LDs), which are thought to originate from the ER (12). To our knowledge, Nsdhl is the first mammalian cholesterol biosynthetic enzyme localized on LDs. Depletion of fatty acids in culture medium reduced the development of LDs and caused Nsdhl redistribution to the ER. Elevation of oleic acid (Ole) in medium induced well-developed LDs with Nsdhl, Ole, oleic acid; PNS, post-nuclear supernatant; TIR, transferrin receptor; PDI, disulfide isomerase; ADRP, anti-adipophilin; aa, amino acid; CHO, Chinese hamster ovary; FCS, fetal calf serum; PBS, phosphate-buffered saline.
and simultaneously caused a reduction in cellular conversion of lanosterol into cholesterol. Human NSDHL with a missense mutation, causing a human embryonic developmental disorder, CHILD syndrome (congenital hemidysplasia with ichthyosis-form nevus and limb defects) (13), could no longer be localized on the surface of LDs. Although the expression of wild-type NSDHL can restore the defective growth of a CHO cholesterol auxotroph, LEK2 (8, 15), and cell cholesterol-deficient medium, the expression of the mutated NSDHL failed to do so. In addition we show that TIP47, a cargo selection protein for mannose 6-phosphate receptors (MPRs) from late endosomes to the trans-Golgi network (14), was localized on LDs positive for Nsdhl; because the expression of NSDHL was previously shown to correct the defective sorting of cation-independent MPR from multivesicular late endosomes to the Golgi in the CHO mutant, LEK2 (8, 15), the observation may provide a link between the surface domains of LDs and late endosomal sorting that depends on cholesterol and TIP47. Together, these results suggest the functional significance of NSDHL localization on the LD surface.

**EXPERIMENTAL PROCEDURES**

**Antibodies**

Rabbit anti-Nedhl antibody was raised against a synthetic peptide corresponding to the C-terminal 14 amino acids of mouse Nedhl and an additional peptide (CERTQFSCRHLEILR) was degased by reductive methylaion and purified on an affinity column of the same peptide immobilized onto thiopropyl-Sepharose 6B through the cysteine residue. Rabbit polyclonal anti-protein disulfide isomerase (PDI) and mouse monoclonal anti-IgG-B clone 1B3 were as described previously (16–18). The following antibodies were purchased: mouse monoclonal anti-adipophilin (ADR) and guinea pig polyclonal anti-human TIP47 N terminus (sa 1–16) (Progen), rabbit polyclonal anti-catalase (Nordic Immunological Laboratories), mouse monoclonal anti-GM130 (clone 35) (BD Transduction Laboratories), anti-VSV-G epitope (mouse monoclonal clone 5D4, Roche Applied Science; rabbit polyclonal, Medical & Biological Laboratories), anti-calnexin (mouse monoclonal clone 37, BD Transduction Laboratories; rabbit polyclonal, Stressgen), mouse monoclonal anti-PDI (Stressgen), and mouse monoclonal anti-γ-tubulin (Sigma).

**Cell Incubation**

Wild-type and mutant CHO cells were cultured in Ham’s F-12 medium with 5% fetal calf serum (FCS), 100 units/ml penicillin, and 10 μg/ml streptomycin (FCS/F-12). The other cells were cultured in 10% FCS/Dulbecco’s modified Eagle’s medium. For microscopy, cells were grown on glass cover slips. Cells were washed with Hank’s balanced salt solution and then incubated as described below. All experiments were done before cells reached confluency. Medium supplements were prepared as follows. Lipoprotein-deficient fetal calf serum (LPDS) was prepared as described elsewhere (19). Delipidated fetal calf serum (DelLS) was prepared using organic solvents as described elsewhere (20) and dialyzed extensively against 0.9% NaCl. Delipidated LPDS or DeLS was used at 2.5 mg of protein/ml in the final medium. Fetal calf serum (DeLS) was prepared using organic solvents as described elsewhere (21). Free fatty acid concentration was determined using NEFA C-test (WAKO). LPDS and DeLS were as described previously (20) and dialyzed extensively against 0.9% NaCl. Delipidated serum albumin (Intergen) as described elsewhere (21). Free fatty acid concentration was determined using NEFA C-test (WAKO). LPDS and DeLS were as described previously (20) and dialyzed extensively against 0.9% NaCl. Delipidated serum albumin (Intergen) as described elsewhere (21). Free fatty acid concentration was determined using NEFA C-test (WAKO).

**Expression Constructs and Cell Transfection**

Human NSDHL—A VSV-G tag was introduced to the C terminus of human NSDHL cDNA subcloned into pBluescript SK– (Stratagene) (8) by a PCR-based method (22) using primers 5'-GCAACACGAGTCTCGAGCC-3' and 5'-CTCTAGAATGCGGCGGAATTC-3'. The construction of cDNA of NSDHL-VSV-G with either of the CHILD syndrome missense mutations (NSDHL (G205S)-VSV-G, NSDHL(A105V)-VSV-G) was done by a PCR-based method (22) using the following pairs of primers: NSDHL(G205S)-VSV-G, NSDHL(A105V)-VSV-G) was done by a PCR-based method (22) using the following pairs of primers: NSDHL(G205S)-VSV-G, 5'-CATGATGAGGAACTGTGTTT-3', 5'-TGCTGACCCGAGCATT-3'. The cDNAs were excised with SstI and NcoI, blunted, and subcloned into the SnoBI site of pBabe-puro expression vector (23).

**Human TIP47**—TIP47 cDNA was amplified from a human cDNA library using the Expand High Fidelity PCR System (Roche Applied Science) with primers 5'-CAGAGACCATGTCGCGCC-3' and 5'-CTCTAGAATGCGGCGGAATTC-3' and was subcloned into a T-vector generated from pBluescript SK– (Stratagene) using the EcoRV site (24). A resulting construct was confirmed by DNA sequencing to harbor in the forward direction a complete open reading frame encoding the wild-type human TIP47 (14). This TIP47 cDNA was excised using ClaI and EcoRI, blunted, and subcloned into the SnoBI site of pBabe-puro.

**Subcellular Fractionation**

All fractionation procedures were done at 4 °C. Cells (150-mm dish) were washed 4 times with PBS and once with homogenization buffer containing protease inhibitors (HB/Cpi: 10 mM Heps-KOH (pH 7.4), 1 mM EDTA, 0.25% sucrose, Complete protease inhibitor mixture (Roche Applied Science)). The cells were scraped in 0.6 M HB/Cpi using a rubber policeman and were homogenized by passing through a 22-gauge x 1 1/2-inch needle 13 times up and down. The homogenate was centrifuged at 500 g for 5 min, and the supernatant was collected as a post-nuclear supernatant (PNS).

For sedimentation gradient centrifugation, the PNS was adjusted to 1.0 M sucrose and loaded onto a linear sucrose gradient (0.3–1.6 M (11 ml) in 10 mM Heps-KOH (pH 7.4)). For floatation gradient centrifugation, the PNS was adjusted to 1.2 M sucrose, 10 mM Heps-KOH (pH 7.4) containing Complete protease inhibitor mixture (Roche Applied Science). The cells were incubated in 0.6 M HB/Cpi using a rubber policeman and were homogenized by passing through a 22-gauge x 1 1/2-inch needle 13 times up and down. The homogenate was centrifuged at 500 g for 5 min, and the supernatant was collected as a post-nuclear supernatant (PNS).

For sedimentation gradient centrifugation, the PNS was adjusted to 1.0 M sucrose and loaded onto a linear sucrose gradient (0.3–1.6 M (11 ml) in 10 mM Heps-KOH (pH 7.4)). For floatation gradient centrifugation, the PNS was adjusted to 1.2 M sucrose, 10 mM Heps-KOH (pH 7.4) containing Complete protease inhibitor mixture, and 2 ml of the PNS was applied on the top of a 1.6 M sucrose cushion (2 ml) placed at the bottom of a centrifugation tube. The PNS was overlaid successively with 2.0 ml each of 0.75, 0.5, 0.25% sucrose containing 10 mM Heps-KOH (pH 7.4). The gradients were centrifuged in an SW41 Ti rotor at 100,000 g for 16 h at 4 °C.

After centrifugation, 12 fractions were collected (bottom = 1). Protein was precipitated from the fractions with trichloroacetic acid and analyzed by SDS-10% PAGE and immunoblotting using monoclonal peroxidase-coupled secondary antibodies followed by detection using Chemiluminescence Reagent Plus (PerkinElmer Life Sciences).

**Sterol Extraction and Thin-layer Chromatography Analyses**

Cells (75 mm² flask) were labeled by incubation in medium (4 ml) containing [1-4C]Cholesterol (10 μCi/ml, Moravek Biochemicals) for 1 h at 37 °C. The cells were then washed with ice-cold PBS (5 ml) 6 times and dissolved in 3 M KOH (3 ml) at room temperature. The samples were transferred to glass conical tubes with a Teflon-coated screw cap (10 ml) and evaporated under N₂ until the volume was reduced to 0.5 ml or less. Methanol (1.5 ml) and pyrogallol (0.1 mg) were added to each sample, and the air phase in the tubes was replaced with N₂. Then the solutions were heated for 2.5 h at 85 °C (26). The nonsaponifiable lipid fraction was extracted 4 times with 4 ml each of petroleum ether. The petroleum ether fraction was then added to 10 ml methanol and evaporated in vacuo.

The extracted nonsaponifiable sterols were analyzed by thin-layer chromatography (TLC) on LKSD preactivated silica gel G plates (Whatman) with methylene chloride/ethyl acetate (97:3 v/v) as the mobile phase (27). Alternatively, sterols were first acetylated overnight in a
mixture of dry pyridine and acetic anhydride (1:1 v/v) and were then analyzed on silica gel G plates that had been preactivated and twice prechromatographed with 10% AgNO₃ in acetonitrile. The silver-imregnated plates were developed using hexane/benzene (65:35 v/v) at 4°C. The radioactivity on the TLC plates was visualized, and migration profiles were drawn using a BAS2000 image analyzer (Fuji). Migration of nonradioactive standard sterols and their acetylated derivatives was determined by spraying the plates with a rhodamine 6G solution (27).

**RESULTS**

**VSV-G-tagged Human NSDHL Was Localized on LDs**—The late part of cholesterol biosynthesis from lanosterol in mammalian cells has previously been localized uniformly over the ER by enzymatic methods (10). Nsdhl is involved in the removal of the two C-4 methyl groups from lanosterol (Fig. 1A), although the exact reaction catalyzed by this enzyme has not been identified (28). We attempted to determine if Nsdhl was indeed localized in the ER. Human NSDHL C-terminally tagged with a VSV-G epitope (NSDHL-VSV-G) was stably expressed in CHO cells. In Western blotting, a single band of 42 kDa was recognized by anti-VSV-G tag antibody in the transfected cells (Fig. 1B). By immunofluorescence, in many cells grown in the normal growth medium containing 5% FCS (FCS/F-12), NSDHL-VSV-G was unexpectedly observed in punctate staining over some diffuse staining in the cytoplasm (Fig. 2A). This punctate staining was lost when cells were grown in LPDS/F-12. Thus, we speculated that the punctate staining was associated with the lipid load to the cells. Indeed, when cells were stained with Sudan III, which stains LDs, the punctate NSDHL-VSV-G staining was closely associated with small LD dots (Fig. 2A).

**Fig. 1. Detection of Nsdhl by Western blotting.** A, the cholesterol biosynthesis step in which Nsdhl is implicated. Nsdhl functions as a C-3 sterol dehydrogenase involved in the complex series of reactions that remove the two C-4 methyl groups from lanosterol (28). HMG-CoA, 3-hydroxy-3-methylglutaryl CoA. B, total cell lysates of CHO cells (lanes 1 and 2) and CHO expressing NSDHL-VSV-G (lane 3) were analyzed by SDS-PAGE followed by immunoblotting using rabbit polyclonal anti-Nsdhl antibody (lane 1) or mouse monoclonal anti-VSV-G antibody (lanes 2 and 3). C, left four lanes, CHO wild-type (WT) or LEX2 mutant cells were cultured for 1 day in LPDS/F-12 (LPDS) or in FCS/F-12 (FCS). Total cell lysates were prepared, and equal amounts (12 μg of protein) of samples were analyzed by SDS-PAGE followed by immunoblotting using anti-Nsdhl antibody. Cholesterol deprivation by incubation in LPDS/F-12 promoted the expression of endogenous Nsdhl in wild-type cells. LEX2 cells lacked Nsdhl expression under either of these conditions. In the other lanes, total lysates of LEX2 cells stably expressing human NSDHL or human NSDHL-VSV-G, F9 cells, and HeLa cells were analyzed by SDS-PAGE followed by immunoblotting using anti-Nsdhl antibody. The masses of these protein molecules calculated from predicted amino acid sequences are as follows: mouse Nsdhl (362 aa), 40.7 kDa; human NSDHL (373 aa), 42.0 kDa; NSDHL-VSV-G (384 aa), 43.2 kDa. D, CHO wild-type cells were cultured for 1 day in F-12 or F-10 medium containing FCS, LPDS or DelS in the presence or absence of additional Ole (50 μg/ml) as indicated. Total cell lysates were prepared, and equal amounts (5.7 μg of protein) of samples were analyzed by SDS-PAGE followed by immunoblotting for Nsdhl, ADRP, and calnexin, as indicated.
thus, were present at much lower levels than those found in undiluted serum. In humans, depending on nutritious states, serum free fatty acids may roughly range between 100 and 700 μEq/liter (29). To confirm the LD localization of NSDHL-VSV-G, the cells stably expressing NSDHL-VSV-G were loaded with a physiologically expected level of Ole (180 μEq/liter = 50 μg/ml; conjugated to bovine serum albumin), the most abundant fatty acid found in human serum (29). Ole administration induced larger LDs. NSDHL-VSV-G was observed concentrated around these induced LDs stained with Sudan III (Fig. 2B).

In contrast, NSDHL-VSV-G did not colocalize extensively with an ER marker, PDI (not shown). However, in cells transiently expressing a high amount of NSDHL-VSV-G, reticular ER-like staining of NSDHL-VSV-G was also observed in cytoplasm (Fig. 2C, NSDHL-VSV-G). Overall distribution of this reticular staining was similar to ER markers (PDI, calnexin) (Fig. 2C and not shown), indicating that some of the overexpressed NSDHL-VSV-G may distribute in the ER also. However, PDI staining apparently avoided the regions close to the surface of LDs, where NSDHL-VSV-G was strongly positive (Fig. 2C, arrows).

NSDHL Was Localized around LDs in Cells Requiring This Enzyme—The localization of endogenous NSDHL was also examined. A rabbit polyclonal antibody raised against the C-terminal 14-amino acid sequence of mouse Nsdhl recognized not only the murine Nsdhl (apparent molecular mass, 39 kDa) in F9 cells but also human NSDHL of higher apparent molecular mass (41 kDa) in HeLa cells (Fig. 1C). This antibody recognized a single band (39 kDa) polypeptide in CHO cells also by immunoblotting (Fig. 1B). When CHO cells were cultured in LPDS/F-12, the expression of the 39 K polypeptide was elevated (Fig. 1C), consistent with this polypeptide being an enzyme involved in cholesterol metabolism (30). This is most likely the feedback regulation of CHO Nsdhl required for cholesterol synthesis under cholesterol depletion. A previously isolated CHO mutant, LEX2, cannot grow in the absence of cholesterol because of deficiency in cholesterol biosynthesis but can be rescued by the expression of human NSDHL (8). By Western blot using the anti-Nsdhl antibody, endogenous Nsdhl was undetectable in

Fig. 2. Human NSDHL expressed in CHO cells was localized around LDs. CHO cells stably (A and B) or transiently (C) expressing NSDHL-VSV-G were incubated for 1 day in FCS/F-12 (A) or in Ole (50 μg/ml)-containing LPDS/F-12 (B and C). The cells were processed for double fluorescence microscopy for NSDHL-VSV-G and LDs (A and B) or NSDHL-VSV-G and PDI (C) using antibodies for the VSV-G antigen and for PDI and Sudan III for LDs. The cells were observed by conventional fluorescence microscopy (BX50 microscope) (A and C) or by laser-scanning confocal microscopy (LSM510) (B). Arrows indicate the close association of NSDHL-VSV-G with LDs (A–C) where PDI was negative (C). N, nucleus. Bars, 20 μm.

Fig. 3. Nsdhl was localized around LDs in cells requiring this enzyme. A and B, CHO wild-type cells were cultured in the presence of additional Ole (50 μg/ml) in FCS/F-12 (A) or LPDS/F-12 (B) for 1 day and processed for double fluorescence microscopy for Nsdhl and LDs using antibody for Nsdhl and Sudan III for LDs. For comparison purposes, images of samples under two different conditions were identically obtained and processed. The arrows indicate the localization of Nsdhl at the surface of LDs. C–F, CHO wild-type cells were cultured in the presence of additional Ole (50 μg/ml) in LPDS/F-12 for 1 day and processed for double fluorescence microscopy for ADRP and Nsdhl (C), Nsdhl (green), and GM130 (red) (D), Nsdhl (green) and TfR (red) (E), or catalase (green) and LDs (red) (F) using antibodies for proteins and Sudan III for LDs. The arrows indicate the colocalization between ADRP and Nsdhl on the LD surface. In D and E, the arrowheads indicate Nsdhl not colocalized with GM130 and TfR, respectively. The arrowheads in F indicate catalase not associated with LDs. G and H, CSG211 (G) or HeLa (H) cells were cultured in the presence of additional Ole (50 μg/ml) in LPDS/Dulbecco’s modified Eagle’s medium for 1 day and processed for double fluorescence microscopy for Nsdhl (green) and LDs (red) using antibody for Nsdhl and Sudan III for LDs. The arrows indicate the localization of Nsdhl on LDs. I, LEX2 cells stably expressing NSDHL-VSV-G were selected by culturing in LPDS/F-12. These cells pretreated with additional Ole (50 μg/ml) in LPDS/F-12 were processed for double fluorescence microscopy for NSDHL-VSV-G (green) and LDs (red) using monoclonal anti-VSV-G antibody and Sudan III, respectively. The arrows indicate the localization of NSDHL-VSV-G on the LD surface. Samples were observed under BX50 (A, B, D, E, G, and H) or LSM510 (C, F, and I). N, nucleus. Bars, 20 μm.
Fractions were collected (A and B) of additional Ole (50 µg/ml) in LPDS/F-12 for 1 day. A PNS was prepared from these cells and subjected to sedimentation sucrose density gradient centrifugation. C, CHO wild-type cells were cultured in the absence of Ole in DeLS/F-10 for 1 day. A PNS was prepared from these cells and subjected to sedimentation sucrose density gradient centrifugation. Fractions were collected (1 = bottom) and processed for SDS-PAGE followed by immunoblotting for Nsdhl, ADRP, calnexin, and TfR, as indicated.

LEX2 cells, whereas expressed human NSDHL or NSDHL-VSV-G in LEX2 cells was clearly recognized by the antibody (Fig. 1C), indicating that LEX2 cells lack the functional Nsdhl protein and that the 39-kDa endogenous polypeptide recognized by the antibody in CHO wild-type cells is indeed the hamster Nsdhl. This antibody was used to detect endogenous Nsdhl in CHO cells loaded with Ole (50 µg/ml). As the result, endogenous Nsdhl was localized at the surface of LDs in immunofluorescence (Fig. 3A).

When CHO cells were loaded with Ole (50 µg/ml) and at the same time depleted of cholesterol by culturing in LPDS/F-12, the expression of endogenous Nsdhl was again elevated (Fig. 1D), whereas the addition of Ole did not elevate the amount of Nsdhl (Fig. 1D). Nsdhl elevated by cholesterol depletion was predominantly observed around LDs rather than in the cytoplasmic ER-like staining (Fig. 3B). ADRP is one of a specific set of proteins that surrounds LDs in a wide array of cell types (31, 32). ADRP expression is induced by Ole along with LD induction (Fig. 1D and Ref. 33). The elevated Nsdhl was well colocalized with the induced ADRP around LDs in CHO cells (Fig. 3C) but not with other organelle markers such as lgp-B (late endosomes/lysosomes, not shown), GM130 (Golgi, Fig. 3D), and transferrin receptors (TfR, early endosomes, Fig. 3E). On the other hand, peroxisomes have been suggested to be sites for cholesterol production other than the ER (34). In adipocytes, peroxisomes are associated with the periphery of LDs (35). However, punctate staining of a peroxisome marker, catalase, was not associated with LDs in CHO cells (Fig. 3F). These results show that up-regulated Nsdhl in cholesterol-depleted cells was mainly localized around LDs. Essentially the same results were obtained with endogenous Nsdhl in mouse epithelial CSG211 cells and in HeLa cells (Fig. 3G and H), indicating that the localization of Nsdhl on LDs is not a phenomenon restricted to only a particular cell type.

When transfected with NSDHL, LEX2 mutant cells can grow in cholesterol-deficient medium. This growth is totally dependent on the expression of NSDHL (8). NSDHL-VSV-G also could restore the growth of LEX2 cells in cholesterol-deficient medium (not shown), showing that this tagged protein is functional. LEX2 cells transfected with NSDHL-VSV-G were selected by culturing in LPDS/F-12. In these selected cells in the growing phase, NSDHL-VSV-G was localized around LDs rather than in the ER-like network (Fig. 3F). The levels of expression of NSDHL-VSV-G were varied among cells, but the majority of NSDHL-VSV-G appeared to be localized around LDs irrespective of the expression levels. These results together with the localization of endogenous Nsdhl elevated in

**Fig. 4.** Nsdhl was co-fractionated with LDs in sucrose density gradient centrifugation. A and B, CHO wild-type cells were cultured in the presence (A) or absence (B) of additional Ole (50 µg/ml) in LPDS/F-12 for 1 day. PNSs were prepared from these cells and subjected to sedimentation (A and B) or floatation (A, B) sucrose density gradient centrifugation. C, CHO wild-type cells were cultured in the absence of Ole in DeLS/F-10 for 1 day. A PNS was prepared from these cells and subjected to sedimentation sucrose density gradient centrifugation. Fractions were collected (1 = bottom) and processed for SDS-PAGE followed by immunoblotting for Nsdhl, ADRP, calnexin, and TfR, as indicated.

endosomes/lysosomes, not shown), GM130 (Golgi, Fig. 3D), and transferrin receptors (TfR, early endosomes, Fig. 3E). On the other hand, peroxisomes have been suggested to be sites for cholesterol production other than the ER (34). In adipocytes, peroxisomes are associated with the periphery of LDs (35). However, punctate staining of a peroxisome marker, catalase, was not associated with LDs in CHO cells (Fig. 3F). These results show that up-regulated Nsdhl in cholesterol-depleted cells was mainly localized around LDs. Essentially the same results were obtained with endogenous Nsdhl in mouse epithelial CSG211 cells and in HeLa cells (Fig. 3G and H), indicating that the localization of Nsdhl on LDs is not a phenomenon restricted to only a particular cell type.

When transfected with NSDHL, LEX2 mutant cells can grow in cholesterol-deficient medium. This growth is totally dependent on the expression of NSDHL (8). NSDHL-VSV-G also could restore the growth of LEX2 cells in cholesterol-deficient medium (not shown), showing that this tagged protein is functional. LEX2 cells transfected with NSDHL-VSV-G were selected by culturing in LPDS/F-12. In these selected cells in the growing phase, NSDHL-VSV-G was localized around LDs rather than in the ER-like network (Fig. 3F). The levels of expression of NSDHL-VSV-G were varied among cells, but the majority of NSDHL-VSV-G appeared to be localized around LDs irrespective of the expression levels. These results together with the localization of endogenous Nsdhl elevated in

**Fig. 5.** Depletion of fatty acids in medium affected Nsdhl localization. CHO wild-type cells were cultured for 1 day in LPDS/F-12 (A–D) or DeLS/F-10 (E–J) in the absence (A–I) or presence (J) of additional Ole (5 µg/ml). The cells were then processed for double immunofluorescence microscopy for Nsdhl (green) and PDI (A, E, F, and J), lgp-B (B and G), TfR (C), GM130 (D and H), or γ-tubulin (I, red). A–D, in the cells cultured for 1 day in LPDS/F-12, Nsdhl was mainly localized in the ER. E, in about 70% of the cells cultured in DeLS/F-10 without additional Ole, Nsdhl was observed in an ER-like distribution (large arrow). F–J, in about 30% of the cells cultured in DeLS/F-10 without Ole, Nsdhl was observed in a cytoplasmic lump (arrows). The arrowheads in H and I indicate the Golgi (GM130) and the microtubule-organizing center, respectively. J, in cells cultured in DeLS/F-10 with additional Ole (5 µg/ml), Nsdhl localized mainly on small LDs and some also in the ER-like staining. N, nucleus. Bars, 20 µm.
FIG. 6. The level of Ole in medium affected cellular cholesterol biosynthesis. CHO wild-type (WT) and LEX2 cells were preincubated for 1 day in DeLS/F-10 in the presence of the indicated concentrations of additional Ole at 37 °C. The cells were then incubated in the presence of [1-\(^{14}\)C]acetate (10 μCi/ml) in the same medium as in the preincubation for 1 h at 37 °C. Sterols were extracted from the cells and analyzed by TLC.
cholesterol-depleted wild-type cells show that Nsdhl was mainly localized around LDs in cells requiring this enzyme.

**Nsdhl Was Cofractionated with LDs in Sucrose Density Gradient Centrifugation**—The association of Nsdhl with LDs was also examined by sucrose gradient centrifugation after cell disruption. LDs from Ole (50 μg/ml)-loaded, cholesterol-depleted CHO cells were recovered in the top fraction (≈12) in both sedimentation and floatation centrifugation, as shown by the unique distribution of ADRP (Fig. 4A). This top fraction was devoid of a membranous ER marker calnexin (Fig. 4A). In both of the gradients, a substantial amount of endogenous Nsdhl that had been elevated by cholesterol depletion was recovered in the top fraction enriched with LDs (Fig. 4A), corroborating that Nsdhl is associated with LDs.

Another substantial part of Nsdhl was always cofractionated with calnexin in denser fractions in both the sedimentation and floatation gradients (Fig. 4A). In the above fluorescence microscopic experiments under the same conditions, reticular ER-like localization of endogenous Nsdhl was not clearly observed (Fig. 3, B–E). Thus, the fractionation results may mean that the association of some Nsdhl with LDs is unstable after cell disruption because of, for example, membrane or cytoskeleton disorganization. However, some Nsdhl in the denser fractions may be localized in the ER together with calnexin, which is reportedly present throughout the ER network (36), or this Nsdhl may be associated with LD-like ER domains, which may correspond to pre-LDs still connected to the ER and/or still poor in both ADRP and stored lipids (thus denser than mature LDs). Although such possibilities cannot be resolved, the fractionation results clearly show that, even after disruption of cells, Nsdhl has an affinity to mature LDs, positive for ADRP and recovered in the top fraction.

**Depletion of Fatty Acids in Culture Medium Affected Nsdhl Localization**—Because Nsdhl was localized around LDs induced by Ole, we next examined the effect of fatty acid depletion in the medium on Nsdhl localization. LPDS is devoid of lipoproteins and, thus, is expected to be free of most esterified fatty acids that are present in lipoproteins. However, free fatty acids that are conjugated to serum proteins such as serum albumin are expected to be present in LPDS. To further deplete fatty acids in medium as completely as possible, we used DeLS depleted with organic solvents (DeLS). In addition, Ham’s F-10 medium devoid of linoleic acid, which is present in F-12 medium (at 0.3 μEq/liter), was used. Thus, LPDS/F-12 medium contained 6.8 μEq/liter free fatty acids derived from LPDS plus 0.3 μEq/liter linoleic acid derived from F-12. DeLS/F-10 medium contained 1.5 μEq/liter fatty acids derived from DeLS.

In CHO wild-type cells cultured in LPDS/F-12 for 1 day, Nsdhl was mainly colocalized with the ER marker, PDI, by microscopy (Fig. 5A). Although minor, there appeared to be a perinuclear overlapping staining between Nsdhl and TTR (Fig. 5C, arrow). The colocalization of Nsdhl with Igp-B or with GM130 was negligible (Fig. 5, B and D). In addition, in a minority (≈5%) of the cells, Nsdhl was observed in a cytoplasm, loosely packed lump (not shown, cf. Fig. 5F, for that observed with DeLS/F-10). The predominant ER localization of Nsdhl was also observed in sucrose density gradient centrifugation; Nsdhl was mostly cofractionated with calnexin (Fig. 4B). However, Nsdhl distribution was well resolved from TTR distribution in sucrose gradient centrifugation (Fig. 4B).

Because of the lack of cholesterol in DeLS, cultivation of CHO wild-type cells in DeLS/F-10 for 1 day led to similar augmentation of Nsdhl to that observed with LPDS/F-12 (Fig. 1D). In these cells cultured in DeLS/F-10, Nsdhl was mostly cofractionated with calnexin in sucrose gradient fractionation (Fig. 4C). Consistent with this, in the majority (≈70%) of the cells cultured in DeLS/F-10, Nsdhl was mainly colocalized with the ER marker PDI by microscopy (Fig. 5E). In addition, under this rigorous depletion of fatty acids, about 30% of total cells appeared somewhat round and unhealthy. In these unhealthy-looking cells, Nsdhl staining was observed as a cytoplasmic lump (usually one lump per cell), positive for PDI (Fig. 5F). The remaining part of the ER in these cells as stained with anti-PDI antibody was not intermingled with the Nsdhl lump but appeared to be disrupted to some extent (Fig. 5F). The Nsdhl lump did not colocalize with the Golgi marker GM130 or with γ-tubulin, which is expected to colocalize with aggresomes, the cytoplasmic aggregates of misfolded proteins (37). Supplementation of DeLS/F-10 medium with Ole (5 μg/ml = 18 μEq/liter) caused concentrated staining of Nsdhl around the small LDs induced by Ole in addition to weak cytoplasmic ER-like Nsdhl staining (Fig. 5J). In addition, Ole (5 μg/ml) addition to fatty acid-depleted medium improved cell growth (not shown) as previously reported (38) and prevented completely the formation of unhealthy-looking cells with the cytoplasmic Nsdhl lump (Fig. 5J). The addition of a higher amount of Ole (50 μg/ml) to DeLS/F-10 resulted in well developed LDs with Nsdhl (not shown), similar to those observed in the presence of Ole (50 μg/ml) in LPDS/F-12 (cf. Fig. 3D).

Together these results clearly indicate that Ole levels in medium can determine not only the extent of LD development but also the Nsdhl distribution. When Ole-induced LDs were present in cells, Nsdhl was able to be distributed mainly on LDs. When LDs were not available, Nsdhl was distributed mainly in the ER. Rigorous depletion of fatty acids caused some unhealthy-looking cells (≈30% of total) in which Nsdhl was distributed to the unidentified, cytoplasmic lump structure.

**The level of Ole in Medium Affected Cholesterol Biosynthesis**—To evaluate the functional significance of the LD localization of Nsdhl, the effect of changing Ole levels in culture medium on cholesterol biosynthesis was examined. Ole addition to DeLS/F-10 did not change the Nsdhl protein level in CHO wild-type cells (Fig. 1D). Nevertheless, because Ole affected the distribution of Nsdhl between LDs and the ER, the Ole level in culture medium might affect Nsdhl activity, if the distribution of Nsdhl on LDs is functionally significant. Nsdhl is involved in the conversion of lanosterol (a C-30 sterol) to cholesterol (a C-27 sterol). After 1 h of pulse-labeling with [1-14C]acetate in wild-type cells newly synthesized C-27 sterols as well as newly synthesized precursor sterols (C-30, C-29, and/or C-28 sterols) were observed (Fig. 6 WT). By contrast, in LEX2 cells, where the Nsdhl function is defective, only the precursor sterols (C-30, C-29, and/or C-28 sterols) were detectable (Fig. 6, LEX2). Changing the levels of Ole in culture medium changed the ratio between the newly synthesized C-27 sterols and the newly synthesized precursor sterols in wild-type cells; in wild-type cells, an increase in Ole to 50 μg/ml in medium caused a decrease in the newly synthesized C-27 sterols and concomitant increase in the newly synthesized precursor sterols that were observed in LEX2 cells (Fig. 6). These results suggest that the localization of Nsdhl on well-developed LDs negatively
regulated the Nsdhl-catalyzed conversion of the precursor sterols into C-27 sterols.

**NSDHL with a Missense Mutation Causing CHILD Syndrome** —Some mutations in the human NSDHL gene cause a human embryonic developmental disorder, CHILD syndrome (MIM 308050), an X-linked dominant, male-lethal trait characterized by an inflammatory nevus with striking lateralization and strict midline demarcation as well as ipsilateral hypoplasia of the body (13). Konig et al. (13) report heterozygous mutations, each of which is causative of typical CHILD syndrome, two independent nonsense mutations (R88X, Q210X) and two independent missense mutations (A105V, G205S). To further estimate the functional significance of intracellular Nsdhl localization, we examined the localization of these missense mutant proteins with a C-terminal VSV-G tag expressed in CHO cells.

We were able to obtain CHO cells stably expressing NSDHL(A105V)-VSV-G. In these cells, NSDHL(A105V)-VSV-G was observed around LDs like wild-type NSDHL (Fig. 7A). The expression of NSDHL(A105V)-VSV-G corrected the defective growth of LEX2 in LPDS/F-12 (not shown), suggesting that the mutant protein is functional at least partially.

In contrast, we could not obtain CHO cells stably expressing NSDHL(G205S)-VSV-G at the microscopic level. The rescue experiments revealed that NSDHL(G205S)-VSV-G cannot restore LEX2 growth in cholesterol-deficient medium (not shown). In transient expression experiments, a much smaller population of CHO cells were found to express NSDHL(G205S)-VSV-G as compared with the cells transfected with the wild-type protein. In these positive cells, NSDHL(G205S)-VSV-G was not observed around LDs but observed as cytoplasmic dots (Fig. 7B).

The cytoplasmic dots of NSDHL(G205S)-VSV-G did not perturb or colocalize with any of such organelle markers as GM130 (Golgi, Fig. 7C), Lgp-B (late endosome/lysosome, not shown), or TIR (early endosome, not shown). Staining with anti-Nsdhl antibody showed that, in cells expressing NSDHL(G205S)-VSV-G, endogenous wild-type Nsdhl was still present around LDs (Fig. 7D, arrowheads), suggesting that endogenous Nsdhl was not perturbed by the expression of NSDHL(G205S)-VSV-G.

**TIP47 Was Associated with LDs Positive for Nsdhl** —We have previously shown that the expression of Nsdhl can correct the abnormal arrest of cation-independent MPR transport/sorting within the multivesicular late endosomes in the CHO mutant, LEX2 (8, 15). It has been reported that TIP47, a protein that functions as a cargo selection device for MPRs from the endocytic pathway to the trans-Golgi network (14), has a high degree of homology to ADRP (43% identical in the amino acid sequence in humans) (39) and can associate with LDs (39, 40). The association of TIP47 with LDs, however, has been somewhat controversial (41). We are particularly interested in this issue because, if TIP47 and Nsdhl, two proteins implicated in late endosomal sorting events, are colocalized on LDs, it may provide further implications regarding the nature of the LD surface as a functional domain.

We thus examined if TIP47 is colocalized with Nsdhl. The anti-human TIP47 antibody (Progen) used here recognized human TIP47 in HeLa cells as a single 47-kDa band in Western blotting (not shown) but did not recognize TIP47 in CHO cells either in Western blotting (not shown) or in immunofluorescence (cf. Fig. 8C, hTIP47 negative cells). This antibody had been raised against the N-terminal 16 amino acid peptide of TIP47, to which no homologous sequence exists in human ADRP (41). Using this antibody, we first examined the localization of endogenous TIP47 in HeLa cells. When permeabilization of cells for immunofluorescence was done with digitonin (a saponin), TIP47 was detected around LDs that were also positive for endogenous Nsdhl (Fig. 8A and B). Notably, however, when permeabilization was done with Triton X-100, this peri-LD staining of TIP47 was virtually lost (not shown). This may explain the discordant results obtained by other groups about TIP47 localization; when saponin was used for cell permeabilization, TIP47 was localized on LDs (39, 40), but when Triton X-100 was used, it failed to be localized on LDs (41).

We also examined the localization of human TIP47 expressed in CHO cells, in which endogenous TIP47 was undetectable with the antibody. The expressed human TIP47 was detected around LDs that were also positive for endogenous Nsdhl (Fig.
containing additional Ole (50 μg/ml) for 1 day and processed for double fluorescence microscopy for TIP47 and NSDHL (A) or TIP47 (green) and LDs (red) (B) using antibodies for proteins and Sudan III for LDs. TIP47 was colocalized with NSDHL on LDs (arrows). C, human TIP47 was transiently expressed in CHO wild-type cells. The cells were pretreated with LPDS/F-12 containing PuGENE 6 transfection mixture for 22 h and then with LPDS/F-12 containing additional Ole (50 μg/ml) for 5 h before fixation. The cells were processed for double immunofluorescence microscopy for human TIP47 and Nsdhl. Expressed human TIP47 was colocalized with Nsdhl (arrows). D, CHO wild-type cells stably expressing human TIP47 were pretreated with LPDS/F-12 for 20 h and then with LPDS/F-12 containing additional Ole (50 μg/ml) for 5 h before fixation. The cells were processed for double fluorescence microscopy for human TIP47 (green) and LDs (red) using anti-TIP47 antibody and Sudan III, respectively. Human TIP47 was localized around LDs (arrows). The cells were observed under BX50. N, nucleus. Bars, 20 μm.

Fig. 8. TIP47 was associated with LDs positive for Nsdhl. A and B, HeLa cells were cultured in LPDS/Dulbecco’s modified Eagle’s medium containing additional Ole (50 μg/ml) for 1 day and processed for double fluorescence microscopy for TIP47 and NSDHL (A) or TIP47 (green) and LDs (red) (B) using antibodies for proteins and Sudan III for LDs. TIP47 was colocalized with NSDHL on LDs (arrows). C, human TIP47 was transiently expressed in CHO wild-type cells. The cells were pretreated with LPDS/F-12 containing PuGENE 6 transfection mixture for 22 h and then with LPDS/F-12 containing additional Ole (50 μg/ml) for 5 h before fixation. The cells were processed for double immunofluorescence microscopy for human TIP47 and Nsdhl. Expressed human TIP47 was colocalized with Nsdhl (arrows). D, CHO wild-type cells stably expressing human TIP47 were pretreated with LPDS/F-12 for 20 h and then with LPDS/F-12 containing additional Ole (50 μg/ml) for 5 h before fixation. The cells were processed for double fluorescence microscopy for human TIP47 (green) and LDs (red) using anti-TIP47 antibody and Sudan III, respectively. Human TIP47 was localized around LDs (arrows). The cells were observed under BX50. N, nucleus. Bars, 20 μm.

8, C and D), further eliminating the possibility that the antibody recognized proteins other than TIP47 on LDs. The colocalization of TIP47 with Nsdhl on LDs may provide a link between the surface domain of LDs and late endosomal sorting functions that depends on cholesterol and TIP47.

DISCUSSION

In the present study, we showed that mammalian Nsdhl is localized around LDs. ER marker proteins appeared to be excluded from around LDs. Thus, Nsdhl localization on LDs is specific. In yeast, some enzymes involved in the late sterol biosynthesis have previously been localized to LDs (42, 43). Our present results suggest that the localization of sterol biosynthesis enzymes extends to LDs in mammals also and that the spatial configuration of sterol biosynthesis may be significantly conserved from yeast to mammals. Functional significance of Nsdhl localization on LDs is suggested by the sterol biosynthesis analysis, the expression experiments of mutated NSDHs, and the colocalization of Nsdhl with functional protein at the LD surface.

LDs are thought to originate from the ER and may even be continuous to the ER to some extent (44). In agreement with this, when LD development was prevented by fatty acid depletion, Nsdhl was predominantly localized in the ER. When Ole was added at a physiological level like in human serum, LDs developed, and Nsdhl was observed predominantly on these LDs. In parallel with this increased Nsdhl localization to well developed LDs, cellular total conversion of precursor sterols (C-30, C-29, and/or C-28 sterols) to C-27 sterols appeared to be decreased. These results suggest that the LD localization of Nsdhl is functionally significant in regulating sterol biosynthesis.

LDs have been considered as depots for lipids including cholesterol as cholesteryl esters (44). However, the present results suggest that the LD is not simply a cholesterol depot but may also be involved in the regulation of sterol biosynthesis. When required in steroidogenic cells, cholesterol is released from LDs via hydrolysis of cholesteryl esters by hormone-sensitive lipase or by similar enzymes stimulated by cAMP. For this hydrolysis, enzyme recruitment onto the LD surface and functions of LD surface proteins are thought to be important. The Nsdhl localization on the LD surface may, thus, provide an opportunity for the coordinated regulation between cholesterol release from storage and cholesterol biosynthesis at the level of the LD surface.

Recently, it has been proposed that caveolin on LDs is a key component in maintaining the cellular cholesterol balance; the presence of high levels of a dominant negative form of caveolin-3 on LDs led to a decrease in cholesterol synthesis (45). However, the mechanism by which this caveolin mutant affects cellular cholesterol biosynthesis has been unclear. The functionally significant localization of Nsdhl on LDs may provide a direct link between cholesterol biosynthesis and a caveolin function at the LD surface.

What could be the mechanisms of the regulation of cholesterol biosynthesis by Nsdhl localization on LDs? It is possible that, because of the differences in the physical environments or the differences in the presence of regulatory factors between the ER membrane and the LD surface, Nsdhl might be enzymatically active on the ER but inactive on LDs. Alternatively, it is also possible that, although Nsdhl activity itself is not changed, the availability of Nsdhl substrates (precursor sterols) to Nsdhl is less when Nsdhl is confined to LDs than when dispersed in the ER. In this latter possibility, depending on the main localization site of Nsdhl, the sterol precursor conversion would take place mainly either in the ER or on LDs; thus, the equilibrium of Nsdhl localization between LDs and the ER may determine not only the total rate but also the intracellular localization of cholesterol biosynthesis. Further experimentation will be required to clarify these possibilities.

We observed that, in the presence of sufficient Ole in medium, Nsdhl was mainly localized on LDs even when Nsdhl activity is required for cell growth. This may mean that the LD localization of Nsdhl is in some way beneficial to cell growth despite the fact that the Nsdhl localization on well developed LDs appeared to reduce the rate of cholesterol biosynthesis. Cholesterol is required for cell growth, but too much cholesterol in cells is apparently toxic to cells (46, 47). Clearly, the coordination of an appropriate amount and appropriate localization of cholesterol production should be important for cell growth and viability. The addition of Ole has been shown to be beneficial to the growth of a variety of cell types (48). Such a beneficial effect of Ole on cells might, in part, reflect the requirement of fatty acids for the coordinated regulation of cholesterol biosynthesis. For example, the low cholesterol level in the ER is important for protein translocation across the ER membrane (3). By directing Nsdhl to LDs, the addition of fatty acids may prevent too much cholesterol biosynthesis in the ER or may redirect the cholesterol biosynthesis site from the ER to...
LDs, thereby exerting a beneficial effect on protein translocation and cell growth.

How cells maintain the low cholesterol level in the ER has yet to be investigated. It has been speculated that the intracellular distribution of cholesterol is essentially governed by its high affinity for sphingolipids (49). The esterification of cholesterol by acyl CoA:cholesterol acyltransferase may also be a mechanism to remove free cholesterol at the site of protein translocation (10). If a substantial amount of cholesterol is produced on LDs and/or LD-like domains on the ER, the existence of a caveolin-mediated cholesterol transport system for newly synthesized cholesterol to the plasma membrane (50) and the ability of caveolin to associate with LDs (45, 51, 52) may provide an efficient mechanism to remove cholesterol from LDs, thereby minimizing the dispersion of newly synthesized cholesterol throughout the ER.

Even in the presence of well developed LDs, one NSDHL mutant with a missense mutation (G205S) causing CHILD syndrome was no longer able to reside on LDs but was instead found as the cytoplasmic dots. This protein was apparently functionally defective, since it failed to restore the growth of LEX2 mutant in cholesterol-deficient medium. In contrast, NSDHL with another CHILD syndrome mutation (A105V) showed primary localization on LDs and was able to restore LEX2 growth. The differences in localization and functionality between these mutated proteins are again consistent with the notion that the Nsdhl localization on LDs is functionally significant. Because both Gly-205 and Ala-105 are conserved in all mammalian enzymes in the cholesterol biosynthetic pathway (28), these results suggest another level of molecular coordination at the LD surface, concerning cholesterol-dependent coordination of NSDHL in LEX2 mutant cells was previously shown to colocalize with Nsdhl on the LD surface. Notably, the expression of a caveolin-mediated cholesterol transport system for newly synthesized cholesterol to the plasma membrane (50) produced on LDs and/or LD-like domains on the ER, the existence of a caveolin-mediated cholesterol transport system for newly synthesized cholesterol to the plasma membrane (50) and the ability of caveolin to associate with LDs (45, 51, 52) may provide an efficient mechanism to remove cholesterol from LDs, thereby minimizing the dispersion of newly synthesized cholesterol throughout the ER.

Acknowledgments—We thank Ishido Miwako for help at the initial stage and Hiroshi Okawara for help in cell stock maintenance.

REFERENCES

1. Lange, Y., and Stock, T. L. (1997) J. Biol. Chem. 272, 13103–13108
2. Bretscher, M. S., and Munro, S. (1993) Science 261, 1290–1291
3. Nilsson, I., Olof-Rekila, H., Sletten, J. P., Johnson, A. E., and von Heijne, G. (2001) J. Biol. Chem. 276, 41749–41754
4. Sugii, S., Reid, P. C., Ohgami, N., Shimada, Y., Maue, R. A., Ninomiya, H., Ohno-Iwashita, Y., and Chang, T. Y. (2003) J. Lipid Res. 44, 1033–1041
5. Mobius, W., Van Donceleaar, E., Ohno-Iwashita, Y., Shimada, Y., Heijne, J. F. H., Slot, J. W., and Geuze, H. J. (2003) Traffic 4, 222–231
6. Ikonen, E. (2001) Curr. Opin. Cell Biol. 13, 470–477
7. Wang, Y., Thiele, C., and Huttner, W. B. (2000) Traffic 1, 952–962
8. Miwako, I., Yamamoto, A., Kudaratsuka, T., Nagayama, K., and Ohashi, M. (2001) J. Cell Sci. 114, 1765–1776
9. Thiele, C., Hannah, M. J., Fahrenholz, F., and Huttner, W. B. (2000) Nat. Cell Biol. 2, 42–49
10. Reinhard, M. P., Billheimer, J. T., Faust, J. R., and Gayler, J. L. (1987) J. Biol. Chem. 262, 9649–9655
11. Morìbous, F. P., Fitzy, B. U., and Glossmann, H. (2000) Trends Endocrinol. Metab. 11, 106–114
12. Murphy, D. J., and Vance, J. (1999) Trends Biochem. Sci. 24, 109–115
13. Konig, A., Happe, R., Bornholdt, D., Engel, H., and Grzeschik, K. H. (2000) Am. J. Med. Genet. 90, 339–346
14. Diaz, E., and Pfeffer, S. R. (1998) Cell 93, 433–443
15. Ohashi, M., Miwako, I., Yamamoto, A., and Nagayama, K. (2000) J. Cell Sci. 113, 2187–2205
16. Yoshimori, T., Semb, T., Takekomi, H., Akagi, S., Yamamoto, A., and Tashiro, Y. (1999) J. Biol. Chem. 274, 15984–15989
17. Ohashi, M., Miwako, I., Nakamura, K., Yamaoto, A., Murata, M., Ohnishi, S., and Nagayama, K. (1999) J. Cell Sci. 112, 1125–1138
18. Uthayakumar, S., and Granger, B. L. (1995) Cell. Mol. Biol. Res. 41, 405–420
19. Ohashi, M., Murata, M., and Ohnishi, S. (1992) Eur. J. Cell Biol. 59, 116–126
20. Cham, B. E., and Knowles, B. R. (1976) J. Biol. Chem. 251, 11784–11789
21. Spector, A. A., and Hoak, J. C. (1969) Anal. Biochem. 30, 297–302
22. Heid, H. W., Moll, R., Schwetlick, I., Rackwitz, H. R., and Keenan, T. W. (1998) J. Lipid Res. 39, 1883–1898
23. Sugii, S., Reid, P. C., Ohgami, N., Shimada, Y., Maue, R. A., Ninomiya, H., Ohno-Iwashita, Y., Shimada, Y., Heijne, J. F. H., Slot, J. W., and Geuze, H. J. (2003) Traffic 4, 222–231
24. Ikonen, E. (2001) Curr. Opin. Cell Biol. 13, 470–477
25. Thiele, C., Hannah, M. J., Fahrenholz, F., and Huttner, W. B. (2000) Nat. Cell Biol. 2, 42–49
26. Nagai, J., Katsuki, H., Nishikawa, Y., Nakamura, I., and Kamihara, T. (1974) J. Biol. Chem. 249, 5802–5807
27. Berry, D. J., and Chang, T. Y. (1982) Biochemistry 21, 573–580
28. Liu, X. Y., Dangel, A. W., Kelley, R. I., Zhao, W., Denny, P., Botcherby, M., Catrannach, B., Peters, J., Hunsicker, P. R., Mallon, A. M., Strivens, M. A., Bate, R., Miller, W., Rhodes, M., Brown, S. D., and Herman, G. E. (1999) J. Lipid Res. 40, 11041–11046
29. Brown, M. S., and Goldstein, J. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9146–9150
30. Brasaemle, D. L., Barber, T., Wolins, N. E., Serrero, G., Blanchette-Mackie, E. J., and Londos, C. (1997) J. Lipid Res. 38, 2249–2263
31. Heid, H. W., Moll, R., Schwetlick, I., Rackwitz, H. R., and Keenan, T. W. (1998) J. Cell Biol. 143, 1883–1898
32. Uthayakumar, S., and Granger, B. L. (1995) Cell. Mol. Biol. Res. 41, 405–420
33. Ohashi, M., Murata, M., and Ohnishi, S. (1992) Eur. J. Cell Biol. 59, 116–126
34. Olivier, L. M., and Krisans, S. K. (2000) J. Biol. Chem. 275, 24351–24355
35. Baumann, O., and Walz, B. (2001) Int. Rev. Cytol. 205, 149–214
36. Johnstone, J. A., Ward, C. L., and Kopito, R. R. (1998) J. Cell Biol. 143, 1883–1898
37. Cattanach, B., Peters, J., Hunsicker, P. R., Mallon, A. M., Strivens, M. A., Bate, R., Miller, W., Rhodes, M., Brown, S. D., and Herman, G. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11041–11046
38. Brasaemle, D. L., Barber, T., Wolins, N. E., Serrero, G., Blanchette-Mackie, E. J., and Londos, C. (1997) J. Lipid Res. 38, 2249–2263
39. Cattanach, B., Peters, J., Hunsicker, P. R., Mallon, A. M., Strivens, M. A., Bate, R., Miller, W., Rhodes, M., Brown, S. D., and Herman, G. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11041–11046
40. Brasaemle, D. L., Barber, T., Wolins, N. E., Serrero, G., Blanchette-Mackie, E. J., and Londos, C. (1997) J. Lipid Res. 38, 2249–2263
45. Pol, A., Luetterforst, R., Lindsay, M., Heino, S., Ikonen, E., and Parton, R. G. (2001) J. Cell Biol. 152, 1057–1070
46. Brown, M. S., and Goldstein, J. L. (1980) J. Lipid Res. 21, 505–517
47. Warner, G. J., Stough, G., Bamberger, M., Johnson, W. J., and Rothblat, G. H. (1995) J. Biol. Chem. 270, 5772–5778
48. Ham, R. G., and McKechnie, W. W. (1979) Methods Enzymol. 58, 44–93
49. Sprong, H., van der Sluijs, P., and van Meer, G. (2001) Nat. Rev. Mol. Cell Biol. 2, 504–513
50. Uittenbogaard, A., Ying, Y., and Smart, E. J. (1998) J. Biol. Chem. 273, 6525–6532
51. Fujimoto, T., Kogo, H., Ishiguro, K., Tauchi, K., and Nomura, R. (2001) J. Cell Biol. 152, 1079–1086
52. Ostermeyer, A. G., Paci, J. M., Zeng, Y., Lublin, D. M., Munro, S., and Brown, D. A. (2001) J. Cell Biol. 152, 1071–1078
Localization of Mammalian NAD(P)H Steroid Dehydrogenase-like Protein on Lipid Droplets
Masato Ohashi, Noboru Mizushima, Yukiko Kabeya and Tamotsu Yoshimori

J. Biol. Chem. 2003, 278:36819-36829.
doi: 10.1074/jbc.M301408200 originally published online July 1, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301408200

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 52 references, 27 of which can be accessed free at
http://www.jbc.org/content/278/38/36819.full.html#ref-list-1