Prp19p is an integral component of the heteromorphic protein complex (the NineTeen complex) in the nucleus, and it is essential for the structural integrity of NineTeen complex and its subsequent activation of the spliceosome. We identified Prp19p, which has never been reported in relation to any function outside of the nucleus, as a member of proteins associated with lipid droplets. Down-regulation of Prp19p expression with RNA interference in 3T3-L1 cells repressed lipid droplet formation with the reduction in the level of expression of perilipin and S3-12. The levels of expression of SCD1 (stearyl-CoA desaturase-1), DGAT-1 (acyl-CoA diacylglycerol acyltransferase-1), and glycerol-3-phosphate acyltransferase were also reduced in Prp19p down-regulated cells, and a significant decrease in triglycerides was observed. Unlike perilipin, which is one of the most extensively studied lipid droplet-associated proteins, Prp19p is not essential for cAMP- and hormone-sensitive lipase-dependent lipolysis pathways, even though Prp19p is a component of the lipid droplet phospholipid monolayer, and down-regulation of Prp19p represses fat accretion significantly. These results suggest that Prp19p or Prp19-interacting proteins during lipid droplet biogenesis in adipocytes may be considered as another class of potential targets for attacking obesity and obesity-related problems.

Lipid droplets are subcellular organelles that function as major energy depots by storing neutral lipids, mainly triacylglycerols (1, 2). Therefore, the biogenesis of a lipid droplet is central to whole body energy homeostasis. In addition to their function as energy depots, lipid droplets appear to have important roles in lipid trafficking in adipocytes, cell signaling, and several important human diseases (3–7). It is thus important to understand the mechanism of deposition and mobilization of cellular neutral lipids. Although the mechanisms of lipid droplet biogenesis are still not entirely clear, it is expected that a set of proteins involved in lipid droplet biogenesis could comprise effective targets for the regulation of the whole body energy homeostasis. Lipid droplets are surrounded by a phospholipid monolayer into which many proteins are embedded (8). These surface proteins on adipocyte lipid droplets include structural proteins, such as PAT (perilipin/ADRP/TIP47) family proteins, S3-12, vimentin, and caveolin-1, and enzymes involved in many aspects of lipid metabolism, such as hormone-sensitive lipase (HSL), acyl-CoA synthetase, lanosterol synthase, CGI-58, and NAD(P)-dependent steroid dehydrogenase-like protein and members of the Rab family of GTPases. Some of these lipid droplet-associated proteins are reported to play important roles in the functions of the lipid droplets (7, 9–13).

In this study, in order to explore proteins that are involved in lipid droplet biogenesis, we investigated the protein composition of lipid droplets isolated from cultured 3T3-L1 adipocytes, and we identified several more lipid droplet-associated proteins using two-dimensional gel electrophoresis and mass spectrometry. Most of the additionally identified proteins are enzymes involved in aspects of lipid metabolism and endoplasmic reticulum or well known membrane trafficking-related proteins.

For unknown proteins, their subcellular localization and the expression patterns during the differentiation of 3T3-L1 adipocytes were investigated. mPrp19p, the mouse homolog of the protein encoded by the Pso4/Prp19 gene in Saccharomyces cerevisiae, is strongly associated with lipid droplets as well as the nucleus. Its expression was elevated during the adipogenesis of 3T3-L1 adipocytes. Moreover, down-regulation of Prp19p expression with RNA interference in 3T3-L1 cells inhibited the expression of lipid droplet-associated proteins (perilipin and S3-12). Also, RNA interference of Prp19p repressed fat accretion and suppressed the expression of SCD1, DGAT-1, and GPAT. Taken together, these results strongly indicate that Prp19p participates in the maturation of lipid droplets and fat storage in differentiating preadipocytes.

**EXPERIMENTAL PROCEDURES**

*Animals—C57BL/6 mice were maintained on a regular light-dark cycle (14 h light, 10 h dark) and kept on a standard laboratory chow diet (4.5% w/w fat). Male animals at an age of 12–16 weeks were used for the experiments.*

*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.*

*The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–4.

1 To whom correspondence should be addressed. Tel.: 82-31-280-5961; Fax: 82-31-899-2595; E-mail: trlee@amorepacific.com.
Cell Culture and Differentiation—Mouse 3T3-L1 (ATCC CL173) preadipocyte cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% calf serum. For the differentiation, the medium was replaced with DMEM containing 10% fetal bovine serum, 10 μg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 1 μM dexamethasone at 2 days post-confluence. After 2 days, the medium was changed to DMEM containing 10 μg/ml insulin and 10% fetal bovine serum, and then the cells were refed every 2 days.

Isolation of Lipid Droplets and Two-dimensional Gel Electrophoresis—Ten days after the initiation of differentiation, lipid droplets were isolated from 20 dishes (100-mm) of 3T3-L1 adipocytes, as described by Schroeder and co-workers (16). Briefly, cells were homogenized in 50 mM NaH2PO4 buffer, pH 7.4, containing 154 mM NaCl, 5 mM MgCl2, and a protease inhibitor mixture (Sigma) followed by centrifugation at 800 g for 10 min. To sediment mitochondria, the resulting supernatant was centrifuged at 5,000 × g for 20 min, followed by a second centrifugation step at 26,000 × g in an SW41Ti rotor (Beckman Coulter, Inc., Fullerton, CA) for 2 h at 4 °C. The floating lipid droplet layers were harvested using a Beckman tube slicer, and the lipid droplet fractions were delipidated with cold acetone overnight at −20 °C followed by centrifugation at 14,000 × g for 30 min at 4 °C. The protein pellet was solubilized in ReadyPrep™ Rehydration buffer (Bio-Rad) by incubation in a bath sonicator at 50 °C for 4–5 h. The first-dimension isoelectric focusing was performed by using precast 24-cm, pH 3–10, IPG strips (Amersham Biosciences) in three steps under a step-hold mode. The second-dimension SDS-PAGE was carried out in an Ettan DALTsix system (Amersham Biosciences).

In Gel Tryptic Digestion—Coomassie-stained protein spots were excised from the gel and destained with 45% acetonitrile in 100 mM ammonium bicarbonate. The resulting gel slices were incubated with 10 mM tris(2-carboxyethyl)phosphine hydrochloride, alkylated by the addition of 50 mM iodoacetamide, and then digested in situ with trypsin (100 ng per band in 50 mM ammonium bicarbonate). The tryptic peptides were extracted using a Branson sonicator in 0.2% trifluoroacetic acid in 5% formic acid. The extracted peptides were concentrated using a SpeedVac concentrator.

Mass Spectrometry—Coomassie-stained protein spots were excised from the gel and in-gel digested with trypsin. HPLC/MS/MS analyses of the resulting tryptic peptides were performed in a LCQ DECA XP plus ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA) coupled online with an inhouse packed capillary column (150 μm × 50 cm, with precolumn flow splitting) Surveyor HPLC system (ThermoFinnigan, San Jose, CA) and an in-house built nanospray source. For protein identification, MS/MS data were searched using BioWorks 3.0 software (ThermoFinnigan, San Jose, CA), a program for matching mass spectrometric information with sequence data bases. Proteins were identified based on matching the MS/MS data with mass values calculated for selected ion series of peptides. The sequences of 11 polypeptides obtained by mass spectrometric analysis were asphyxiated with carbon dioxide, and tissues were harvested and immediately frozen in liquid nitrogen. The tissues were extracted in a solution of pH 7.4 containing 20 mM HEPES, 250 mM sucrose, 4 mM EDTA, 0.1% Triton and protease inhibitor mixture. 3T3-L1 cells were lysed in RIPA buffer (PBS, pH 7.4, containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor mixture). Forty micrograms of proteins were resolved on 10% NuPAGE gels run in the MES buffer system (Invitrogen) and transferred to nitrocellulose membranes according to the manufacturer’s protocol. Immunoreactive proteins were revealed by enhanced chemiluminescence with ECL + (Amersham Biosciences). Blots were quantified using an ImageMaster (Amersham Biosciences).

Subcellular Fractionation—Adipocytes were washed three times with ice-cold PBS and scraped into homogenization buffer (50 mM HEPES, pH 7.4, 255 mM sucrose, 1 mM EDTA) containing a protease inhibitor mixture (Sigma). After homogenization with a glass homogenizer for 20 strokes, the homogenate was centrifuged at 1,000 × g for 10 min into supernatant 1 and pellet. The pellet was homogenized in 10 mM Tris buffer, pH 7.5, containing 300 mM sucrose, 1 mM EDTA, and protease inhibitor mixture and centrifuged 5,000 × g for 5 min. The remaining pellet was the nuclear fraction. Supernatant 1 was removed and centrifuged at 5,000 × g for 20 min to yield the pellet (mitochondria fraction) and supernatant 2. Supernatant 2 was centrifuged at 26,000 × g for 2 h to sediment the microsomal fraction. The final supernatant was called cytosol. The floating lipid droplet layers were extracted with 1% SDS and 1% Triton-containing buffer by

**Mouse Prp19p Is a New Lipid Droplet-associated Protein**

Cloning of Mouse Prp19—Mouse Prp19 cDNA was amplified with the primers 5′-ATGTCCCTGATCTGCTCGATCTCCA-3′ and oligo(dT) (Stratagene, La Jolla, CA) using poly(A)+ mRNA prepared from 3T3-L1 cells 10 days after the initiation of differentiation. RT-PCR from the 3T3-L1 cells amplified a 1.6-kb DNA fragment that encoded a protein that contained all 11 polypeptides obtained by mass spectrometric analysis. The sequence matched the previously reported sequence for *Mus musculus Prp19* (GenBank™ accession number NM_134129).

Antibodies—Polyclonal antibody to Prp19 was raised in rabbits and affinity-purified against peptide TTERKKRGK by LabFrontier (Seoul, Korea). The antibody to perilipin was purchased from Research Diagnostics Inc. (Flanders, NJ); the antibodies to poly(ADP-ribose) polymerase, PPAR-γ, and C/EBP-α were from Santa Cruz Biotechnology (Santa Cruz, CA); the antibodies to calnexin and FAS were from BD Biosciences; the antibody to OXphos complex IV was from Molecular Probes (Eugene, OR); the antibodies to cyclin A was from Upstate Biotechnology, Inc. (Lake Placid, NY); the antibody to lamin B was from Santa Cruz Biotechnology; and the antibody to SCD-1 was from Alpha Diagnostic International, Inc. (San Antonio, TX).

Protein Preparation and Immunoblotting—C57BL6 mice were asphyxiated with carbon dioxide, and tissues were harvested and immediately frozen in liquid nitrogen. The tissues were extracted in a solution of pH 7.4 containing 20 mM HEPES, 250 mM sucrose, 4 mM EDTA, 0.1% Triton and protease inhibitor mixture. 3T3-L1 cells were lysed in RIPA buffer (PBS, pH 7.4, containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor mixture). Forty micrograms of proteins were resolved on 10% NuPAGE gels run in the MES buffer system (Invitrogen) and transferred to nitrocellulose membranes according to the manufacturer’s protocol. Immunoreactive proteins were revealed by enhanced chemiluminescence with ECL + (Amersham Biosciences). Blots were quantified using an ImageMaster (Amersham Biosciences).

Subcellular Fractionation—Adipocytes were washed three times with ice-cold PBS and scraped into homogenization buffer (50 mM HEPES, pH 7.4, 255 mM sucrose, 1 mM EDTA) containing a protease inhibitor mixture (Sigma). After homogenization with a glass homogenizer for 20 strokes, the homogenate was centrifuged at 1,000 × g for 10 min into supernatant 1 and pellet. The pellet was homogenized in 10 mM Tris buffer, pH 7.5, containing 300 mM sucrose, 1 mM EDTA, and protease inhibitor mixture and centrifuged 5,000 × g for 5 min. The remaining pellet was the nuclear fraction. Supernatant 1 was removed and centrifuged at 5,000 × g for 20 min to yield the pellet (mitochondria fraction) and supernatant 2. Supernatant 2 was centrifuged at 26,000 × g for 2 h to sediment the microsomal fraction. The final supernatant was called cytosol. The floating lipid droplet layers were extracted with 1% SDS and 1% Triton-containing buffer by
**Mouse Prp19p Is a New Lipid Droplet-associated Protein**

rocking at room temperature for 1 h. The suspension was then warmed up to 37 °C, vortexed vigorously, and immersed eight times for 15 s in a bath sonicator.

**Immunofluorescence Microscopy**—3T3-L1 adipocytes were fixed with 3% formaldehyde in PBS, permeabilized with 70% ethanol, and incubated with anti-Prp19p (1.4 μg/ml) or anti-perilipin (diluted 1:100) antisera in a microscopy buffer (2% BSA and 0.1% Triton X-100 in PBS), followed by Alexa 488-conjugated donkey anti-rabbit IgG (Molecular Probes, Inc., Eugene, OR) and Alexa 594-conjugated goat anti-guinea pig IgG; the same specimen was stained with Sudan III (Wako, Japan) dissolved in 70% ethanol to visualize lipid droplets. DNA was stained with 4’,6-diamidino-2-phenylindole. In some experiments, lipid droplets were stained by Oil red O (Sigma). The specimens were observed with a Zeiss Axiosplan-2 microscope. Image acquisition and post-processing were performed with Northern Eclipse software (Empix, Mississauga, Ontario, Canada). For studying the effect of leptomycin on intracellular localization of Prp19p, differentiated adipocytes were treated with or without 20 nM leptomycin B (Sigma) for 8 h.

**Modification of Prp19p Expression in 3T3-L1 Cells through Transfection with Either pcDNA3.1-mPrp19 or pSilence 2.1-U6 Puro-mPrp19 RNAi**—RNAi directed against the mouse Prp19p gene was designed using Silencer pre-designed RNAi (Ambion, Inc., Austin, TX) and subcloned into the BamHI/HindIII site of pSilencer 2.1-U6 puro (Ambion, Inc., Austin, TX). The following oligonucleotides were used: RNAi-1, sense template 5’-GATCCCGCTGGAACAAACACT-3’ and antisense template 5’-GATCCGCTGGACAACAACT-3’; RNAi-2, sense template 5’-GATCCGACGTCTACGACATCAACATTTTCAAGAGAATTGTGCTGAGCTGCTCTTTTTTGGAAA-3’ and antisense template 5’-GATCCGCTGGACAACAACT-3’; RNAi-3, sense template 5’-GATCCGACGTCTACGACATCAACATTTTCAAGAGAATTGTGCTGAGCTGCTCTTTTTTGGAAA-3’ and antisense template 5’-GATCCGCTGGACAACAACT-3’. To produce Prp19p-overexpressing preadipocytes, mouse Prp19p (mPrp19) cDNA fragment was obtained by RT-PCR by using 3T3-L1 poly(A) RNA and the following primers: 5’-ATGCCTCATCGACATCAACATTTTCAAGAGAATTGTGCTGAGCTGCTCTTTTTTGGAAA-3’ and 5’-ACTACTGAGATGAGGCCAGAACACTGT-3’.

The mPrp19 cDNA was subcloned into the XabI and BamHI sites of the pCDNA3.1 expression vector (Invitrogen). The sequences of pSilencer 2.1-U6 puro-mPrp19 RNAi and pcDNA3.1-mPrp19 were verified by DNA sequencing.

3T3-L1 preadipocytes were transfected with either pcDNA3.1, pcDNA3.1-mPrp19, pSilencer 2.1-U6 puro Negative Control (Ambion, Inc., Austin, TX), or pSilencer 2.1-U6 puro-mPrp19 RNAi using Lipofectamine (Invitrogen). Cells stably expressing Prp19p were selected by resistance to geneticin at 0.5 mg/ml culture medium. For down-regulation of mPrp19 expression, cells transfected with pSilencer 2.1-U6 puro-mPrp19 RNAi vector were selected with puromycin (1.5 μg/ml) for 7 days. The RNAi-3 construct could efficiently knock down Prp19p protein expression. To minimize for clone-specific effects, each study was performed with pools of three Prp19p down-regulated clones.

**Real Time Quantitative RT-PCR**—Total RNA was extracted with TRizol (Invitrogen) according to the manufacturer’s instructions. The pre-designed primers and probe sets of SREBP-1c, aP2, glycerol-3-phosphate acyltransferase, diacylglycerol O-acyltransferase 1, S3-12, SCD1, perilipin, and glycerolaldehyde-3-phosphate dehydrogenase were obtained from Applied Biosystems (assay identifications are as follows: Mm00550338_m1, Mm00445880_m1, Mm00515643_m1, Mm00833328_m1, Mm00491061_m1, Mm00558672_m1, Mm00772290_m1, and Mm99999915_q1). The reaction mixture was prepared using a Quantitect probe PCR kit (Qiagen, GMBH, Germany) according to the manufacturer’s instructions. Reaction and analysis were performed using the Rotor-Gene 3000 system (Corbett Research, Sydney, Australia). All reactions were done in triplicate. The amount of mRNA was calculated by the comparative C_T method.

**Lipid Analysis**—Cellular triglyceride content was determined by extracting cells with 2:1 chloroform/methanol onto ammonium sulfate-impregnated Silica Gel H thin layer chromatography plates. Plates were developed in 90:10:1 hexane/diethyl ether/formic acid and charred at 160 °C for 1–2 h. Spots corresponding to the various lipid classes were quantitated by densitometry using a computing densitometer from GE Healthcare; relative spot densities were calculated using ImageQuant software (GE Healthcare) and compared with lipid standards resolved on the same plates. Intracellular lipid accumulation was also determined by Sudan II staining. The cells were washed twice with ice-cold PBS, fixed with 4% formaldehyde in PBS for 20 min on ice, and stained with 0.5% (w/v) Sudan II (Wako, Japan) in 60% (v/v) isopropyl alcohol for 1 h at room temperature. After staining, the cells were washed with 70% ethanol to remove excess stain. Stained oil droplets in the cells were dissoloved in isopropyl alcohol containing 4% (v/v) Nonidet P-40 and spectrophotometrically measured at an absorbance of 490 nm.

**Lipolysis**—3T3-L1 adipocytes were incubated in a low glucose DMEM medium (Invitrogen) containing 2% (w/v) fatty acid-free BSA with or without 10 μM isoproterenol. Glycerol content of the incubation medium was determined using a colorimetric assay (GPO-Trinder; Sigma). Protein content was determined using a BCA protein assay (Pierce).

**Fatty Acid Oxidation**—3T3-L1 adipocytes placed in a 12-well plate were washed and incubated in low glucose DMEM (Invitrogen) containing 2% (w/v) fatty acid-free BSA, 0.3 mM l-carnitine, and 3[H]palmitic acid (3 μCi/well). Excess [3H]palmitic acid in the medium was removed twice by trichloroacetic acid precipitation. The supernatant was extracted twice with chloroform/methanol (2:1) and then counted for [3H]O production.

**Statistical Analysis**—Data are presented as mean ± S.D. Two-tailed Student’s tests were used to calculate statistical significance.
**RESULTS**

*Identification of Prp19p as a New Lipid Droplet-associated Protein*—To explore proteins that are involved in lipid droplet biogenesis, we isolated lipid droplets from cultured 3T3-L1 adipocyte cells collected at 10 days after the initiation of differentiation, and we identified the proteins associated with lipid droplets using two-dimensional gel electrophoresis and tandem mass spectroscopy (Table 1). One of the identified proteins was mPrp19p, the mouse homolog of the protein encoded by the S. cerevisiae *PSO4/PRP19* gene. We amplified the murine *Prp19* cDNA from 3T3-L1 adipocytes using RT-PCR in order to use to confirm the localization of Prp19p to lipid droplets and to study Prp19p expression and function.

*Analysis of Prp19p Expression*—To confirm the association of Prp19p on the membrane of lipid droplets, we performed immunofluorescence assays in differentiated 3T3-L1 adipocytes. Prp19p was strongly detected on the surface of lipid droplets, we performed immunofluorescence assays in differentiated 3T3-L1 adipocyte cells and confirmed the association of Prp19p to lipid droplets and to study Prp19p expression and function.

*Mouse Prp19p Is a New Lipid Droplet-associated Protein*—To confirm the association of Prp19p to lipid droplets, we performed immunofluorescence assays in differentiated 3T3-L1 adipocytes. Prp19p was strongly detected on the surface of lipid droplets, we performed immunofluorescence assays in differentiated 3T3-L1 adipocytes and confirmed the association of Prp19p to lipid droplets and to study Prp19p expression and function.

*Expression Pattern of Prp19p According to Its Subcellular Localization in 3T3-L1 Adipocytes*—Because Prp19p was detected both on the lipid droplet and nucleus in differentiated adipocytes, we suspected that there was the potential cross-talk between events at the lipid droplet and events in the nucleus. To determine whether Prp19p shuttles between the cytosol and the nucleus, 3T3-L1 adipocytes were treated with or without leptomycin B, nuclear export inhibitor, for 8 h. Localization of Prp19p was unaffected by leptomycin B treatment (Fig. 2A). This result suggests that Prp19p does not shuttle between the cytosol and the nucleus. Next, we examined whether the expression level of Prp19p is increased in the nuclear fraction as well as the lipid droplet during the adipocyte differentiation. As shown Fig. 2B, the expression of Prp19p protein in the lipid droplet was increased during the adipocyte differentiation, whereas that of Prp19p in the nuclear fraction was not affected by the differentiation stage.

*The Effect of Down-regulation of Prp19p on Fat Accumulation in 3T3-L1 Cells*—To determine whether Prp19p is involved in lipid droplet biogenesis, we established stable 3T3-L1 cell lines with either pCDNA3.1-mPrp19 or p-mPrp19 RNAi for overexpression or down-regulation of the mouse *Prp19* gene, respectively (Fig. 3A). Although cells transfected with the control vector exhibited normal lipid droplet accumulation, stable 3T3-L1 cells transfected with p-mPrp19 RNAi formed

---

**Table 1**

Proteins associated with isolated 3T3-L1 adipocytes lipid droplets

This table shows lipid droplet-associated proteins identified by mass spectrometry. Lipid droplets were isolated as described from 3T3-L1 cells 10 days after the initiation of differentiation. The proteins were separated by two-dimensional gel electrophoresis and processed for identification.

| Group                        | Spot | pI  | Mass  | Identification                                                                 | GI no. | Previously found in lipid droplet preparations | Ref. |
|------------------------------|------|-----|-------|--------------------------------------------------------------------------------|-------|-----------------------------------------------|------|
| **Known lipid droplet-associated proteins** | 1    | 5.43| 61.20 | Perilipin                                                                        | 13385312 | Yes                                         | 17, 18 |
|                              | 2    | 5.12| 53.80 | Vimentin                                                                          | 55408   | Yes                                          | 18, 19 |
|                              | 3    | 5.14| 66.90 | Protein-disulfide isomerase A4 precursor, protein ERp-72                        | 119531  | Yes                                          | 4, 20 |
|                              | 4    | 5.44| 80.00 | BIP; glucose-regulated 78K protein                                               | 109893  | Yes                                          | 20–22 |
|                              | 5    | 5.75| 47.30 | TIP47                                                                             | 13385312| Yes                                          | 20–22 |
| **Lipid metabolism**         | 6    | 7.8 | 84.00 | Hormone-sensitive lipase                                                          | 6754552 | Yes                                          | 13, 23 |
|                              | 7    | 6.37| 58.50 | Alcohol dehydrogenase 2                                                          | 6753036 | Yes                                          | 11    |
|                              | 8    | 5.75| 84.40 | Lanosterol synthase                                                               | 22122469| Yes                                          | 4, 13, 20|
|                              | 9    | 8.79| 34.50 | Short-chain dehydrogenase/reductase member 1                                      | 13278172| Yes                                          | 4     |
|                              | 10   | 8.52| 34.78 | Diaphorase I                                                                      | 19745150| Yes                                          | 4, 13, 20|
|                              | 11   | 6.49| 47.90 | CGI-like protein (hydrolase or acyltransferase domain)                           | 27721963| No                                           |       |
|                              | 12   | 8.12| 74.40 | Long-chain acyl-CoA synthetase 4                                                 | 6172341 | Yes                                          | 4, 13, 20|
| **Membrane traffic**         | 13   | 6.06| 55.70 | Sorting nexin 6                                                                   | 27754031| No                                           |       |
|                              | 14   | 5.35| 33.69 | N-Ethylmaleimide sensitive fusion protein attachment protein a (SNAP)            | 13385392| Yes                                          | 4     |
|                              | 15   | 6.15| 57.47 | Synaptic vesicle membrane protein VAT-1                                           | 23623337| No                                           |       |
|                              | 16   | 6.1  | 24.10 | Rab14                                                                             | 18390233| Yes                                          | 4     |
|                              | 17   | 8.25| 23.40 | Rab5c                                                                             | 18606182| Yes                                          | 4     |
|                              | 18   | 6.27| 23.50 | Rab7                                                                              | 6679599 | Yes                                          | 4     |
|                              | 19   | 5.98| 31.17 | Unknown protein (vacuolar assembly/sorting protein DID4 domain)                  | 21321251| No                                           |       |
|                              | 20   | 6.12| 55.15 | Guanosine diphosphate (GD) dissociation                                           | 20984325| No                                           |       |
| **Miscellaneous**            | 21   | 4.67| 67.80 | Calnexin                                                                          | 6671664 | Yes                                          | 11    |
|                              | 22   | 6.24| 61.20 | EHD2                                                                              | 20072042| Yes                                          | 11    |
|                              | 23   | 5.78| 68.90 | Ribophorin1                                                                       | 16359229| Yes                                          | 11    |
|                              | 24   | 6.01| 23.98 | Tumor protein D54                                                                 | 12850393| Yes                                          | 13    |
|                              | 25   | 6.4  | 58.76 | Rho GAP (Rho-like GTPase)                                                        | 13879250| Yes                                          | 4     |
|                              | 26   | 6.64| 31.06 | Unknown protein                                                                    | 14119698| No                                           |       |
|                              | 27   | 6.5  | 54.10 | PRP19/PSO4 homolog                                                                | 26338912| No                                           |       |
|                              | 28   | 5.62| 57.68 | RuvB-like protein 2 (AAA family protein)                                         | 6755382 | Yes                                          | 4     |
significantly fewer lipid droplets, as determined by Oil red O staining after 10 days of differentiation (Fig. 3B). The Prp19p down-regulated cells showed a ~50% decrease in intracellular neutral lipid content when compared with the control cells (Fig. 3C). The other two Prp19 RNAi constructs, which did not reduce the expression of Prp19p, did not affect the content of intracellular neutral lipid (data not shown). In contrast, overexpression of Prp19p resulted in no discernible increase in lipid droplet number or neutral lipid content (Fig. 3, B and C). Also, overexpression of Prp19p had no effect on the rate of the triglyceride accumulation during adipocyte differentiation (data not shown). These results indicate that Prp19p is necessary but not stimulatory for the maturation of lipid droplets and fat storage in differentiating preadipocytes.

The Effect of Down-regulation of Prp19p on Adipogenic Marker Genes and Fatty Acid Oxidation—To gain insight into the mechanisms by which down-regulation of Prp19p represses fat accretion, we measured protein and mRNA expression of adipogenic marker genes in Prp19p down-regulated cells relative to control cells. Adipogenic potential appeared normal in
Prp19p is a New Lipid Droplet-associated Protein

A

B

FIGURE 2. Expression pattern of Prp19p according to its subcellular localization. A, effect of leptomycin B on cellular localization of Prp19p was examined. Differentiated 3T3-L1 cells were treated with or without 20 nm leptomycin B (LMB) for 8 h. Cells were fixed and stained with anti-Prp19p (green). Nucleus was stained by 4′,6-diamidino-2-phenylindole (blue). The cells were observed with a confocal microscope. Bar, 10 μm. Each experiment was repeated three times, and representative results are shown. B, 3T3-L1 adipocytes at various stages of differentiation were harvested, and whole cell lysate or equal cell equivalents of nuclear and lipid droplet extracts were prepared as described under “Experimental Procedures.” 40 μg of cell lysate or equal cell equivalents of nuclear and lipid droplet extracts were separated by gel electrophoresis and processed for immunoblotting with the indicated antibodies. The results shown are representative of three independent experiments.

Prp19p Is Not Directly Involved in the cAMP-, HSL-, and Perilipin-dependent Lipolysis Pathways—To determine the involvement of Prp19p on the cAMP-, HSL-, or perilipin-dependent lipolysis pathways, we compared the basal and isoproterenol-stimulated lipolytic activities of Prp19p down-regulated cells with those of control cells. In Prp19p down-regulated cells, the basal and isoproterenol-stimulated lipolysis decreased by 35 and 25%, respectively, when compared with the control cells. However, between Prp19p down-regulated cells and the control cells, the difference in the increased ratio of the isoproterenol-stimulated lipolysis over the basal lipolysis was not much (Fig. 6). The above findings indicate that although down-regulation of Prp19p represses fat accretion significantly, Prp19p is not directly involved in the cAMP-, HSL-, and perilipin-dependent lipolysis pathways.

DISCUSSION

We identified mouse Prp19p, a 504-amino acid protein with a calculated molecular mass of 54 kDa encoded by murine Prp19 gene, as a new member of proteins associated with lipid droplets isolated from 3T3-L1 adipocytes. In yeast and human, Prp19p has pleiotropic functions in DNA recombination and error-prone repair (24, 25). Prp19p is an integral component of the heteromeric protein complex (the NineTeen complex). This complex is essential for activation of a spliceosome that influences the efficiency of DNA repair via splicing of pre-mRNAs of intron-containing repair genes (26–29). Prp19p makes numerous protein-protein contacts with the NineTeen complex and spliceosome components and is required for the structural integrity of NineTeen complex and its subsequent activation of the spliceosome. Prp19p contains three recognized protein motifs as follows: a WD-40 repeat domain at its C terminus, a U-box at its N terminus, and a predicted coiled-coil domain between the two former motifs. These motifs are required for its tetramerization and scaffolding role for protein-protein interactions with other proteins (30). To date, the reported functions of Prp19p are limited to functions in the nucleus. The role of Prp19p has never been reported in relation to any function outside of nucleus. However, based on its structure and function as an essential scaffold of numerous protein-protein interactions in the nucleus, we speculated that Prp19p might also have some functions in the formation and/or stabilization of lipid droplets through utilization of its capacity to make a number of protein-protein interactions.

Prp19p was abundantly expressed both in adipose tissues and brain tissue (Fig. 1C). The high level expressions of Prp19p in white and brown adipose tissues are not surprising considering its association with the membrane of lipid droplets (Fig. 1, A and B). However, its high level expression in brain tissue is notable. Immunofluorescence assays in PC12 cells showed that Prp19p was not only detected on the nucleus but also on the cytoplasm (data not shown). More studies are needed to investigate the exact molecular function of Prp19p in various cell types. Prp19p expression increased during the differentiation of 3T3-L1 preadipocytes into adipocytes (Fig. 1D). Down-regulation of Prp19p in 3T3-L1 cells reduced the intracellular triglyceride content by 50%. Lipogenic enzymes, such as SCD1 and its downstream triacylglycerol synthesis enzymes, DGAT-1 and
GPAT, were also reduced. Moreover, Prp19p influenced the levels of the expression of the lipid droplet-associated proteins (perilipin and S3-12). It is not easy to explain how a reduction of Prp19p expression reduced downstream regulatory genes for fat storage, such as SCD-1, DGAT-1, perilipin, and S3-12. These effects may be due to the reduction of the Prp19p expression in the nucleus. However, as shown Fig. 2, the expression levels of Prp19 in the nucleus were not affected by the differentiation stage. Moreover, Prp19p did not shuttle between the cytosol and the nucleus. These results support the idea that Prp19p may have a different function according to its subcellular localization in 3T3-L1 adipocytes. Furthermore, Prp19p is not known as a transcription factor but only known as a scaffold of protein complex for activation of the spliceosome that influences the efficiency of DNA repair. If down-regulation of Prp19p influences gene expression through down-regulation of the spliceosome activity, it might have equal influences on the expression of upstream and downstream lipogenic genes and adipogenic transcription factors. However, it does not exert significant influence on the upstream lipogenic genes and adipogenic transcription factors. From these results and based on the well known function of Prp19 as an essential scaffold of numerous protein-protein interactions, we can speculate that down-regulation of Prp19p in 3T3-L1 cells hinders fat storage in differentiating preadipocytes by disturbing the protein-protein interactions that are necessary for maturation and integrity of lipid droplets. This defect in maturation and integrity of lipid droplets might influence the expression levels of downstream regulatory genes for fat storage such as SCD1, DGAT-1, and GPAT, without significant influence on the upstream lipogenic genes. However, we could not provide an exact molecular mechanism for this partly because of poorly understood lipid droplet biogenesis. It is also still uncertain whether these effects of a reduction in the Prp19p are due to its effects only in the lipid droplet or both in the nucleus and lipid droplet. Further studies, such as identifying Prp19p-associated proteins in lipid droplets to compare the effects after specific inhibition of such associations, are needed to determine the precise functions of Prp19p in triacylglycerol packaging and storage in lipid droplets.

Ntambi and co-workers (31) also reported that thiazolidinediones, PPAR-γ agonists, selectively repressed the expression of SCD1 with no significant effect on other genes linked to adipocyte differentiation. Therefore, although thiazolidinediones enhanced adipocyte conversion, they concomitantly caused a dramatic decrease in cell size and lipid droplet compared with the control 3T3-L1 cells. SCD1 inhibition has been suggested as a new target for the treatment of obesity and related metabolic disorders (32–34). SCD1 is a key rate-limiting enzyme in the synthesis of unsaturated fatty acids, major components of triacylglycerols, from long-chain saturated fatty acids. Its regulation can influence a variety of physiological vari-

FIGURE 3. The effect of down-regulation of Prp19p on lipid droplet biogenesis in 3T3-L1 cells. A, Prp19 protein levels in 3T3-L1 cells transfected with control pSilencer 2.1-U6 puro negative control or pCDNA3.1 vectors or with pSilencer 2.1-U6 puro-mPrp19 RNAi or pCDNA3.1-mPrp19 vectors for down-regulation or overexpression of Prp19p, respectively. B, Oil red O staining for triglycerides in transfected 3T3-L1 cells after differentiation for 10 days. C, intracellular triglyceride levels in transfected 3T3-L1 cells differentiated for 10 days. Data shown are means ± S.D. and were obtained from two independent experiments carried out in triplicate (***, p < 0.001).
Reduction in SCD-1 activity results in accumulation of saturated long-chain fatty acids, which has direct influence on the fatty acid synthesis and/or β-oxidation. SCD1-specific antisense oligonucleotide inhibitors reduced de novo fatty acid synthesis, decreased expression of lipogenic genes, and increased expression of genes promoting energy expenditure in liver and adipose tissues (33). We investigated the effect of SCD1 down-regulation in Prp19p down-regulated cells on fatty acid synthesis and β-oxidation. No significant difference was found in the protein expression of FAS (Fig. 4A). The increase of the β-oxidation level was statistically significant but very moderate at 8% (Fig. 5). It is not clear that the 8% increase in β-oxidation alone is sufficient to explain the 50% decrease in triacylglycerol accumulation in Prp19p down-regulated cells. Another possibility is that there are small changes in the lipogenic genes, which cannot be detected with conventional molecular biology tools, which cumulatively account for the 50% decrease in triacylglycerol accumulation. Nonetheless, these results show that down-regulation of Prp19p expression with RNAi in 3T3-L1 cells represses fat accretion, at least in part via repression of expression of SCD1 and its downstream triacylglycerol synthesis enzymes, DGAT-1 and GPAT, and a slight increase in β-oxidation level without a significant effect on other lipogenic genes.

Perilipin is one of the most extensively studied lipid droplet-associated proteins (35). It plays a role as a barrier for HSL...
access to the triacylglycerols in lipid droplets. Perilipin needs to be polyphosphorylated by protein kinase A for translocation of HSL to the lipid droplets and stimulation of lipolysis (36). Consistent with the role of perilipin as a barrier to lipolysis, adipocytes from perilipin-null mice have an elevated basal rate of lipolysis compared with adipocytes from wild-type mice, but they fail to respond maximally to lipolytic stimuli (37, 38). On the contrary, the Prp19p down-regulated adipocyte cells have 35% decreased basal lipolytic activity when compared with the control cells and can fully respond to lipolytic stimuli (Fig. 6). These results indicate that Prp19p is not involved in the cAMP-, HSL-, or perilipin-dependent lipolysis pathways.

In summary, we identified Prp19p as a member of proteins associated with the phospholipid monolayer of lipid droplets, and we also found that Prp19p expression increased during the differentiation of 3T3-L1 preadipocytes to adipocytes. We have also shown that Prp19p is necessary for the maturation of lipid droplets and fat storage in differentiating preadipocytes. Down-regulation of Prp19p with RNAi in 3T3-L1 cells repressed lipid droplet biogenesis via repression of intracellular fat accretion and expression of SCD1, DGAT-1, and GPAT without significant effects on other genes linked to adipocyte differentiation. Although it is not clear if it can adequately account for the 50% decrease in triacylglycerol accumulation, there was also an 8% increase of β-oxidation in Prp19p down-regulated cells. Even though Prp19p is an important component of the lipid droplet phospholipid monolayer for lipid droplet biogenesis, unlike perilipin, Prp19p is not essential for cAMP- and hormone-sensitive lipase-dependent lipolysis pathways. These results suggest that Prp19p or Prp19p-interacting proteins during lipid droplet biogenesis in adipocytes could be another class of potential targets for attacking obesity and obesity-related problems.

REFERENCES

1. Zweytick, D., Athenstaedt, K., and Daum, G. (2000) Biochim. Biophys. Acta 1469, 101–120
2. Murphy, D. J. (2001) Prog. Lipid. Res. 40, 325–438
3. Le Lay, S., Ferre, P., and Dugail, I. (2004) Biochem. Soc. Trans. 32, 103–106
4. Liu, P., Ying, Y., Zhao, Y., Mundy, D. I., Zhu, M., and Anderson, R. G.
Mouse Prp19p Is a New Lipid Droplet-associated Protein

(2004) J. Biol. Chem. 279, 3787–3792
5. Igal, R. A., Rhoads, J. M., and Coleman, R. A. (1997) J. Pediatr. Gastroenterol. Nutr. 25, 541–547
6. Cole, N. B., Murphy, D. D., Grider, T., Rueter, S., Brasaemle, D., and Nussbaum, R. L. (2002) J. Biol. Chem. 277, 6344–6352
7. Yamaguchi, T., Omatsu, N., Matsushita, S., and Osumi, T. (2004) J. Biol. Chem. 279, 30490–304907
8. Tauchi-Sato, K., Ozeki, S., Houjou, T., Taguchi, R., and Fujimoto, T. (2002) J. Biol. Chem. 277, 44507–44512
9. Brown, D. A. (2001) Curr. Biol. 11, R446–R449
10. Londos, C., Sztalryd, C., Tansey, J. T., and Kimmel, A. R. (2005) J. Biol. Chem. 280, 19146–19155
11. Wolins, N. E., Quaynor, B. K., Skinner, J. R., Schoenfish, M. J., Tzekov, A., and Bickel, P. E. (2005) J. Biol. Chem. 280, 31190–31199
12. Fujimoto, Y., Itabe, H., Sakai, J., Makita, M., Noda, J., Mori, M., Higashi, Y., Kojima, S., and Takano, T. (2004) Biochim. Biophys. Acta 1644, 47–59
13. Brasaemle, D. L., Dolios, G., Shapiro, L., and Wang, R. (2004) J. Biol. Chem. 279, 46835–46842
14. Cohen, A. W., Razani, B., Schubert, W., Williams, T. M., Wang, X. B., Iyengar, P., Brasaemle, D. L., Scherer, P. E., and Lisanti, M. P. (2004) Diabetes 53, 1261–1270
15. Robenek, M. J., Severs, N. J., Schlattmann, K., Plenz, G., Zimmer, K. P., Troyer, D., and Robenek, H. (2004) FASEB J. 18, 866–868
16. Atshaves, B. P., Storey, S. M., McIntosh, A. L., Petrescu, A. D., Lukyusytova, O. I., Greenberg, A. S., and Schroeder, F. (2001) J. Biol. Chem. 276, 25324–25335
17. Greenberg, A. S., Egan, J. J., Wek, S. A., Garty, N. B., Blanchette-Mackie, E. J., and Londos, C. (1991) J. Biol. Chem. 266, 11341–11346
18. Blanchette-Mackie, E. J., Dwyer, N. K., Barber, T., Coxey, R. A., Takeda, T., Rondinone, C. M., Theodorakis, J. L., Greenberg, A. S., and Londos, C. (1995) J. Lipid Res. 36, 1211–1226
19. Franke, W. W., Hergt, M., and Grund, C. (1987) Cell 49, 131–141
20. Umlauf, E., Czaszar, E., Moertelmaier, M., Schuetz, G. J., Parton, R. G., and Prohaska, R. (2004) J. Biol. Chem. 279, 23699–23709
21. Miura, S., Gan, J. W., Brzostowski, J., Parisi, M. J., Schultz, C. J., Londos, C., Oliver, B., and Kimmel, A. R. (2002) J. Biol. Chem. 277, 32253–32257
22. Than, N. G., Sumei, B., Belleye, S., Berki, T., Szekeres, G., Janaky, T., Szigeti, A., Bohn, H., and Than, G. N. (2003) Eur. J. Biochem. 270, 1176–1188
23. Szatalryd, C., Xu, G., Dorward, H., Tansey, J. T., Contreras, J. A., Kimmel, A. R., and Londos, C. (2003) J. Cell Biol. 161, 1093–1103
24. Grey, M., Dusterhoft, A., Henriques, J. A., and Brendel, M. (1996) Nucleic Acids Res. 24, 4009–4014
25. Mahajan, K. N., and Mitchell, B. S. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10746–10751
26. Makarova, O. V., Makarov, E. M., Umlauf, H., Will, C. L., Gentzel, M., Wilm, M., and Luhrmann, R. (2004) EMBO J. 23, 2381–2391
27. Chan, S. P., and Cheng, S. C. (2005) J. Biol. Chem. 280, 1029–1037
28. Chen, C. H., Yu, W. C., Tsao, T. Y., Wang, L. Y., Chen, H. R., Lin, J. Y., Tsai, W. Y., and Cheng, S. C. (2002) Nucleic Acids Res. 30, 1029–1037
29. Chan, S. P., Kao, D. I., Tsai, W. Y., and Cheng, S. C. (2003) Science 302, 279–282
30. Ohi, M. D., Vander Kooi, C. W., Rosenberg, J. A., Ren, L., Hirsch, J. P., Chazin, W. J., Walz, T., and Gould, K. L. (2005) Mol. Cell. Biol. 25, 451–460
31. Kim, Y. C., Gomez, F. E., Fox, B. G., and Ntambi, J. M. (2000) J. Lipid Res. 41, 1310–13106
32. Ntambi, J. M., Miyazaki, M., Stoehr, J. P., Lan, H., Kendzierski, C. M., Yandell, R. S., Song, Y., Cohen, P., Friedman, J. M., and Attie, A. D. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 11482–11486
33. Dobrzyn, A., and Ntambi, J. M. (2005) Prostaglandins Leukot. Essent. Fatty Acids 73, 35–41
34. Jiang, G., Li, Z., Liu, F., Ellsworth, K., Dallas-Yang, Q., Wu, M., Ronan, J., Esau, C., Murphy, C., Szalkowski, D., Bergeron, R., Doebber, T., and Zhang, B. B. (2005) J. Clin. Investig. 115, 1030–1038
35. Tansey, J. T., Szatalryd, C., Hlavin, E. M., Kimmel, A. R., and Londos, C. (2004) IUBMB Life 56, 379–385
36. Souza, S. C., Muliro, K. V., Liscum, L., Lien, P., Yamamoto, M. T., Schaffer, J. E., Dollah, G. E., Wang, X., Kraemer, F. B., Obin, M., and Greenberg, A. S. (2002) J. Biol. Chem. 277, 8267–8272
37. Tansey, J. T., Szatalryd, C., Gruia-Gray, J., Roush, D. L., Zee, J. V., Gavrilova, O., Reitman, M. L., Deng, C. X., Li, C., Kimmel, A. R., and Londos, C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6494–6499
38. Castro-Chavez, F., Yechoor, V. K., Saha, P. K., Martinez-Botas, J., Wooten, E. C., Sharma, S., O’Connell, P., Taegtmeyer, H., and Chan, L. (2003) Diabetes 52, 2666–2674