Oligomerization of the Murine Fatty Acid Transport Protein 1*

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The 63-kDa murine fatty acid transport protein 1 (FATP1) was cloned on the basis of its ability to augment fatty acid import when overexpressed in mammalian cells. The membrane topology of this integral plasma membrane protein does not resemble that of polytopic membrane transporters for other substrates. Western blot analysis of 3T3-L1 adipocytes that natively express FATP1 demonstrate a prominent 130-kDa species as well as the expected 63-kDa FATP1, suggesting that this protein may participate in a cell surface transport protein complex. To test whether FATP1 is capable of oligomerization, we expressed functional FATP1 molecules with different amino- or carboxyl-terminal epitope tags in fibroblasts. These epitope-tagged proteins also form apparent higher molecular weight species. We show that, when expressed in the same cells, differentially tagged FATP1 proteins co-immunoprecipitate. The region between amino acid residues 191 and 475 is sufficient for association of differentially tagged truncated FATP1 constructs. When wild type FATP1 and the non-functional s250a FATP1 mutant are co-expressed in COS7 cells, mutant FATP1 has dominant inhibitory function in fatty acid uptake assays. Taken together, these results are consistent with a model in which FATP1 homodimeric complexes play an important role in cellular fatty acid import.

Evidence is emerging that proteins are important mediators and/or regulators of trafficking of long chain fatty acids (LCFAs) across the plasma membrane of cells (for a review see Ref. 1). The first member of the fatty acid transport protein family, FATP1, was identified in a functional screen for adipocyte proteins that facilitate fatty acid import into cells (2). This integral plasma membrane protein is a member of a large family of related proteins in prokaryotic and eukaryotic organisms (3). The exact mechanism by which these proteins mediate LCFA import is poorly understood. Given its plasma membrane localization, high level of expression in tissues with efficient LCFA import, and the ability of overexpressed FATP1 to facilitate LCFA uptake into cells, this protein was initially proposed to function as a transporter or shuttle for moving LCFA across the plasma membrane. Gain and loss of function studies suggest that FATP1 and its yeast ortholog also have very long chain acyl-CoA synthetase activity (4–8). Based on site-directed mutagenesis experiments, FATP1 has been proposed to contain separable domains that function in transport and esterification, respectively (6, 8).

Experimental evidence for the membrane topology of FATP1 supports a topology model with one transmembrane domain near the amino terminus of the protein (9). The extreme amino terminus of the protein faces the extracellular space, and the carboxyl terminus faces the cytosol. The amino-terminal 190 amino acids are hydrophobic, and three stretches of sequence within this region are independently capable of directing integral membrane association of an enhanced green fluorescent protein reporter. Given that the amino terminus of FATP1 is extracellular and residues 191–257 are not membrane-associated and likely face the cytosol, we predict that FATP1 contains at least one transmembrane domain in the region between residues 1 and 190. Between residues 258 and the carboxyl terminus, at least two domains are capable of associating peripherally with the membrane, but no additional transmembrane or integral membrane domains have been identified.

Overall, this proposed membrane topology does not resemble those of polytopic membrane transporters for hydrophilic substrates. Many classic transporters are predicted to have transmembrane domains consisting of primarily α-helical structures of 17 or more amino acids that span the phospholipid bilayer. Depending on the specific transporter, between 4 and 12 transmembrane domains are thought to form a three-dimensional channel through which the substrate passes. Unfavorable interactions between hydrophilic substrates and the hydrophobic core of the membrane may be minimized in such a model. However, it is unclear whether such a structure would be utilized for transport of amphipathic substrates, such as long chain fatty acids, that readily adsorb into membranes. Rather fatty acids may move across bilayers in direct contact with membrane phospholipids.

On the other hand, some proteins that have been shown to facilitate transport of substrates when expressed in mammalian cells have few transmembrane domains. For example, a type II single membrane-spanning protein, the rbAT heavy chain, was expression-cloned on the basis of its ability to promote dibasic and neutral amino acid transport (10–12). This protein participates in a heterodimeric amino acid transport complex in which it is disulfide-linked to a polytopic membrane transport protein (light chain) (13).

Similarly, FATP1 may participate in an oligomeric cell surface fatty acid transport complex. The goal of the present study was to test the hypothesis that FATP1 molecules are capable of dimerizing and thereby extend our understanding of the mech-

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§ The abbreviations used are: LCFA, long chain fatty acid; BODIPY 3823, 4,4-difluoro-5-methyl-4-bora-3a,4a-diaz-a-s-indacene-3-dodecanonic acid; HA, hemagglutinin; HRP, horseradish peroxidase; CMV, cytomegalovirus; NGFR, nerve growth factor receptor; PBS, phosphate-buffered saline; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.

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FATP1 Oligomerization

anism of action of FATP proteins. Here we use several approaches to show that FATP1 forms detergent-resistant dimers that play a functional role in LCFA import into mammalian cells.

EXPERIMENTAL PROCEDURES

Materials—We obtained BODIPY 3823 (4,4-difluoro-5-methyl-4-bora-3a,4a-diazas-indacene-3-dodecanoic acid) from Molecular Probes, Protease Complete and Protein A- and Protein G-agarose from Roche Molecular Biochemicals, mouse monoclonal anti-FATP antibody (M2) from Sigma-Aldrich Inc., mouse monoclonal anti-HA antibody (HA.11) from Covance Inc., mouse monoclonal anti-Myc antibody (SC-40) from Santa Cruz Biotechnology, horseradish peroxidase (HRP)-coupled IgG from Jackson ImmunoResearch Laboratories, Inc., and Renaissance Western blot chemiluminescence reagents from PerkinElmer Life Sciences. Antibodies to native FATP1 sequences were generated as previously described (2, 14). Cell culture reagents were from Invitrogen. NIH 3T3 fibroblasts, 3T3-L1 adipocytes and COS7 cells (American Type Culture Collection) were grown as previously described (15, 16) using cell culture reagents from Invitrogen and Sigma.

Expression Constructs—PCR was used to insert amino-terminal HA, amino-terminal Myc, and carboxyl-terminal FLAG tags into ΔU3FATP1 or ΔU3FATP1s250a mutant retroviral constructs (14). Recombinant baculovirus was generated and used to transduce cells as previously described (17). PCR was used to clone fragments of FATP (amino acid residues 1–190, 191–475, or 476–646) into pCMVtag2 and pCMVtag3 vectors (Stratagene) and used to transfect COS7 cells using LipofectAMINE Plus (Invitrogen). To optimize expression of constructs containing residues 191–475 and residues 476–646, N-acetyl-Leu-Leu-norleucinal (Calbiochem) was included in the media at 10 μg/ml after transfection. PCR was also used to generate an HA-tagged FATP1c and a Myc-tagged FATP1c250a construct in the vector pcDNA3.1.

Fatty Acid Uptake Assays—Parental and retroviral-transduced NIH 3T3 cells were assayed for fatty acid uptake as previously described (14). Flow cytometric analysis of samples of 10⁶ propidium iodide-negative (live) cells was carried out in triplicate using a BD Biosciences FACSCalibur. For assessment of FATP truncation constructs, 2 × 10⁸ COS7 cells were plated in 10-cm dishes on the day prior to transfection. Transfections of DNA (20 μg) with LipofectAMINE Plus (Invitrogen) were performed according to the manufacturer’s instructions. Immunoprecipitations were performed 24 h after transfection.

For assays of dominant inhibitory function of mutant FATP, 8 × 10⁶ COS7 cells were plated in 6-cm dishes. The following day, cells were transfected with LipofectAMINE 2000 and 10 μg of total DNA, consisting of 1 μg of a construct for inactivated nerve growth factor receptor (NGFR) (18), 3 μg of FATP1, and 0–6 μg of FATP1s250a or 6–0 μg of empty vector (pcDNA1.1). 48 h after transfection, cells were stained for NGFR using an E-phycocyanin-conjugated anti-NGFR antibody (Chromaprobe, 1:50 in PBS with 2% inactivated fetal bovine serum for 20 min at 4°C) and assayed for uptake of BODIPY 3823. BODIPY fluorescence (FL1 channel) of phycocyanin-positive cells (FL2 channel) was determined by flow cytometry.

Protein Isolation—For Western blot analysis of FATP protein expression in various cell types, total protein or total post-nuclear membranes were isolated as described (19). Proteins were quantified by bicinchoninic acid assay (Pierce Chemical Co.).

Immunoprecipitations—All manipulations were carried out at 4°C. Cells were washed in PBS and lysed in TNET (50 mM Tris, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1 μM phenylmethylsulfonyl fluoride, 1× Protease Complete) containing iodoacetamide (100 μM; Nuclei were pelleted by centrifugation at 1000 × g for 10 min. Primary antisera were incubated with post-nuclear supernatants for 1 h at the following dilutions: oHα (1:100), oFLAG (1:750), and oMyc (1:50). Protein G-agarose beads were added, and samples were incubated for an additional 45 min. Beads were washed three times with TNET and once with TBS, and bound proteins were eluted by boiling for 5 min in 2× Laemmli sample buffer.

Western Blot Analysis—Proteins were separated by SDS-PAGE (7.5–12% acrylamide) and transferred to nitrocellulose (Schleicher and Schuell, 0.2-μm pore). Primary antibodies for Western analysis were used at the following dilutions: 1:6000 FATP1 (directed against amino acids 1–455), 1:1000 oHα, 1:450 oMyc, and 1:500 oFLAG. Detection was performed using HRP-coupled secondary antibodies and Renaissance Western blot chemiluminescence reagents (PerkinElmer Life Sciences).

Sucrose Density Gradient Isolation of FATP1 Oligomers—NIH 3T3 cells expressing FATPnHA were treated with 100 μM iodoacetamide.

Cells were lysed in TNET and nuclei were pelleted by centrifugation at 1000 × g for 10 min. Molecular weight standards were added, and the protein sample was loaded onto a 25–35% continuous sucrose gradient. Gradients were centrifuged at 108,000 × g for 2 h. Fractions (0.3 ml) were collected, and FATP protein was analyzed by immunoprecipitation and Western blotting. Chromatography standards (Sigma) included as markers in each gradient were ovalbumin (40 kDa), bovine serum albumin (66 kDa), β-galactosidase (116 kDa), alcohol dehydrogenase (150 kDa), and apoferritin (443 kDa). Fractions were analyzed for markers by SDS-PAGE and Coomassie Blue staining.

RESULTS

Western analysis of membrane proteins from 3T3-L1 adipocytes probed with an antibody directed against FATP1 residues 455–470 reveals the expected 63-kDa FATP1 as well as a higher molecular mass species of ~130 kDa (Fig. 1). Neither species is observed in pre-adipocytes, which do not express FATP1. A second antiserum directed against FATP1 residues 628–640 also recognizes proteins of both sizes (data not shown). These findings suggest that FATP1 participates in an oligomeric complex that is resistant to SDS denaturation. Although it is possible that FATP1 interacts with one or more other unidentified proteins in a hetero-oligomeric complex, the apparent size of this higher molecular weight species is consistent with participation of FATP1 in a homo-oligomeric complex. Lack of alteration in the intensity of this higher molecular weight species under non-reducing conditions (data not shown) suggests that it does not represent a disulfide-linked oligomer.

To study the oligomeric state of FATP1, we generated and characterized several epitope-tagged FATP1 constructs in the ΔU3 retroviral vector. An amino-terminal HA tag (FATPnHA) or a carboxyl-terminal FLAG tag (FATPcFLAG) was inserted in-frame with FATP1 coding sequences. Constructs were transfected into 293GPG packaging cells to generate retrovirus for high level stable transduction of NIH 3T3 cells. Western blot analysis of cellular lysates shows similar levels of expression of wild type and mutant epitope-tagged forms of FATP1 using an antibody to native FATP1 residues for detection (Fig. 2A). Western analysis using epitope tag antibodies reveals that these antisera are specific and do not recognize other tags. In addition, we generated cells that co-express FATPnHA and FATPcFLAG. Each of these constructs was tested for function in fatty acid uptake assays using the fluorescent fatty acid
Compared with native FATP1, FATPnHA has similar activity for fatty acid uptake. As has been previously observed, FATPcFLAG is less efficient in promoting fatty acid uptake, possibly due to perturbation of protein conformation upon placement of the epitope tag at the carboxyl terminus (9). Cells co-expressing both FATPnHA and FATPcFLAG have total FATP levels comparable to cells expressing either construct alone and comparable levels of fatty acid uptake.

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FLAG cells were mixed prior to immunoprecipitation. Immunoprecipitation was observed from lysates prepared using a variety of
another in a homo-oligomeric complex. Co-immunoprecipitation experiments from cells that co-express FATPnHA and FATPcFLAG. Specifically, we sought to determine whether
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weight species containing FATP1. To confirm the specificity of this interaction, we performed immunoprecipitations from cells that co-express FATPnHA and FATPcFLAG in parallel with immunoprecipitations from cells that express only one or the other construct (Fig. 5B). In addition, we mixed Triton X-100 lysates from FATPnHA-expressing and FATPcFLAG-expressing cells prior to immunoprecipitation. We observed efficient co-immunoprecipitation only when the differentially tagged proteins are synthesized within the same cells but not when lysates from FATPnHA cells and from FATPcFLAG cells are mixed. These observations show that FATP1 molecules are capable of associating in an oligomer that contains at least two molecules of FATP1. Moreover, our results suggest that dimer formation occurs during the biosynthesis of FATP1 or that formation of dimers requires intact cellular membrane structure.

In an effort to identify the region of FATP involved in dimerization, we generated a series of constructs containing domains of FATP1. Constructs containing residues 1–190 encompass the hydrophobic amino-terminal region that is integrally associated with membranes. Constructs containing residues 191–475 include the putative ATP-interacting site that is essential for FATP1 function as well as a region that is peripherally associated with membranes. Constructs containing residues 476–646 encompass a region of FATP1 that is soluble and projects into the cytosol (9). In each instance, these sequences were fused in-frame with an amino-terminal HA or FLAG tag to facilitate detection. Constructs were co-expressed in COS7 cells and assayed for co-immunoprecipitation. Constructs containing residues 1–190 could not be expressed even in the presence of proteasomal inhibitors (data not shown) possibly due to misfolding or cytotoxicity. Thus, the contribution of the amino terminus of FATP1 to dimerization remains undetermined. On the other hand, constructs containing residues 191–475 or 476–646 were detected by immunoprecipitation and Western blot analysis (Fig. 6). The HA-(191–475) and FLAG-(191–475) constructs co-immunoprecipitate, as detected by immunoprecipitation with anti-HA and Western blot with anti-FLAG. Neither HA-(191–475) nor FLAG-(191–475) co-immunoprecipitated with either epitope-tagged form of residues 476–646. Also, HA-(476–646) and FLAG-(476–646) did not co-immunoprecipitate. These results suggest that the region of FATP1 encoded by residues

**Fig. 5.** FATPnHA and FATPcFLAG co-immunoprecipitate. A, NIH 3T3 cells expressing FATPnHA and FATPcFLAG and parental NIH 3T3 cells were lysed in 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA containing 1% Triton X-100 (lanes 1–4), 1% CHAPS (lanes 5 and 6), or 1% SDS (lanes 7 and 8). Lysates were immunoprecipitated (IP) with α-HA and α-FLAG antibodies and analyzed by Western blotting (WB) using both antibodies. Detection was performed using HRP-coupled secondary antibody and chemiluminescence. This blot is representative of three independent experiments. B, NIH 3T3 cells expressing FATPnHA (lanes 3 and 4), FATPcFLAG (lanes 5 and 6), or both proteins (lanes 1 and 2) were lysed in 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 200 mM iodoacetamide and immunoprecipitated with α-HA (lanes 1, 3, 5, and 7) or α-FLAG (lanes 2, 4, 6, and 8) antibodies. In lanes 7 and 8, lysates from FATPnHA cells and FATPcFLAG cells were mixed prior to immunoprecipitation. Immunoprecipitated proteins were separated by SDS-PAGE, and analyzed by Western blot analysis with α-HA or α-FLAG antibodies, HRP-coupled secondary antibody and chemiluminescence. This blot is representative of three independent experiments.

FATPnHA cells were also separated on continuous sucrose-density gradients. Fractions were analyzed by immunoprecipitation and Western blot using an anti-HA antibody. The location of FATP1 in these fractions was compared with protein markers loaded within the same gradients. We observed a biphase distribution of FATP with one peak occurring between the ovalbumin and albumin markers (40 and 66 kDa) and a second peak between the β-galactosidase and alcohol dehydrogenase markers (116 and 150 kDa) (Fig. 4). This biphase distribution of FATP1 is consistent with the existence of both a monomeric and oligomeric form (~130 kDa) of FATP1 within cells.

To explore the molecular composition of the high molecular weight species containing FATP1, we performed immunoprecipitation experiments from cells that co-express FATPnHA and FATPcFLAG. Specifically, we sought to determine whether the differentially tagged FATP1 proteins interact with one another in a homo-oligomeric complex. Co-immunoprecipitation was observed from lysates prepared using a variety of detergents (Fig. 5A), suggesting that the higher molecular

**Fig. 6.** FATP1 residues 191–475 contain a dimerization domain. COS7 cells were transiently transfected with constructs for FATP1 amino acids 191–475, or 476–646, each containing either an HA or FLAG epitope tag. Immunoprecipitations were performed with anti-HA or anti-FLAG antibodies, followed by Western blot analysis with anti-HA or anti-FLAG. HRP-coupled secondary antibody and chemiluminescence were used for detection. This blot is representative of three independent experiments.
191–475 contains a dimerization domain that is sufficient for self-association.

To assess the contribution of FATP1 oligomers to fatty acid uptake, we expressed wild type and mutant FATP1 sequences in the same cells. We used an FATP1 mutant with a single conservative amino acid substitution at residue 250 that does not affect biosynthesis or targeting of the protein to the plasma membrane (14). When expressed at levels similar to that of the HA-tagged wild type FATP1, the Myc-tagged FATP1s250a mutant lacks the ability to facilitate fatty acid uptake (Fig. 7, A and B), consistent with our prior studies. However, wild type and mutant Myc-tagged FATP1 co-immunoprecipitate equally well with wild type HA-tagged FATP1 (Fig. 7C). Thus, although conservative mutation of residue 250 impairs fatty acid uptake, the s250a FATP1 mutant has dominant inhibitory function in FA import.

**Fig. 7.** s250a FATP1 mutant has dominant inhibitory function in FA import. A, NIH 3T3 cells were transduced with retroviruses encoding wild type and mutant (s250a) FATP1 with an amino-terminal HA or Myc tag, respectively. Total protein (50 µg) from parental fibroblasts (lane 1, 3T3) or transduced cells (lane 2, FATPnHA; lane 3, FATPnMycs250a) were separated by SDS-PAGE (8%) and analyzed by Western blot. Antiserum directed against the native FATP1 (αFATP, amino acids 455–70) and the epitope tags (αHA, αMyc) were used with horseradish peroxidase (HRP)-coupled secondary antibodies and chemiluminescence for detection. B, parental and transduced cells were assayed for uptake of the fluorescent fatty acid analog BODIPY 3823 for 1 min at 37 °C as described under “Experimental Procedures.” For each sample, flow cytometric analysis of 10⁷ propidium iodide-negative (live) cells was assessed. Plot shows average median fluorescence of at least six independent measurements ± S.E. Analysis of variance was determined using a two-tailed t test (equal variance). *, p < 0.001 for FATPnHA cells versus 3T3 cells; **, p < 0.001 for FATPnMycs250a cells versus FATPnHA cells. C, NIH 3T3 cells expressing FATPnHA (lanes 2 and 7), FATPnMycs250a (lanes 3 and 8), FATPnHA and FATPnMyc (lanes 4 and 9), or FATPnHA and FATPnMycs250a (lanes 5 and 10) were lysed in 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 200 mM iodoacetamide and immunoprecipitated with α-HA (lanes 1–5) or α-Myc (lanes 6–10) antibodies. Immunoprecipitated proteins were separated by SDS-PAGE and analyzed by Western blot analysis with α-HA or α-Myc antibodies, HRP-coupled secondary antibody, and chemiluminescence. This blot is representative of three independent experiments. D, COS7 cells were transfected with indicated amounts of wild type (FATPnHA) and s250a mutant (FATPnMycs250a) FATP1 DNA. Cells were assayed for LCFA uptake and analyzed by flow cytometry as described under “Experimental Procedures.” The graph shows average median fluorescence of three independent measurements ± S.E. Analysis of variance was determined using a two-tailed t test (equal variance). *, p < 0.01; **, p < 0.001; ***, p < 0.001 for cells transfected with both mutant and wild type FATP1 as compared with cells transfected with wild type FATP1 only. These results are representative of three independent experiments.
transport function of FATP1, this residue is not critical for formation of FATP1 dimers.

To delineate whether the FATP1s250a mutant has dominant inhibitory effects on fatty acid transport, we transiently transfected COS7 cells with a constant amount of wild type FATPnHA and increasing amounts of FATPnMycs250a (Fig. 7D). We observed a dose-dependent decline in fatty acid uptake as the ratio of mutant to wild type FATP1 DNA sequences increased, suggesting that the mutant has dominant inhibitory effects on fatty acid transport. This finding shows that FATP1 participates in oligomers that function in fatty acid uptake in live cells.

**DISCUSSION**

The FATPs are a family of integral membrane proteins that play an important role in cellular lipid homeostasis. To date, no crystallographic data is available for any family members, although topology studies of FATP1 suggest that this protein has a single transmembrane domain. Determining the oligomeric state for FATPs is important for understanding the molecular mechanism by which FATP family proteins function. Based on the ability of our anti-FATP1 antisera to recognize higher molecular weight species in addition to the 63-kDa FATP1, we hypothesized that FATP1 participates in a cell surface fatty acid transport complex. In the present study, we provide the first evidence that FATP1 forms detergent-resistant dimers that play a functional role in fatty acid transport. Epitope-tagged full-length FATP1 molecules co-immunoprecipitate when they are co-expressed in cells. Moreover, co-immunoprecipitation experiments using different domains of FATP1 demonstrate that residues 191–475 are sufficient for FATP1 dimerization. Based on our data, we propose in Fig. 8 a model for the structure of an FATP1 oligomer.

Studies from our laboratory (14, 20) and others (4) have shown that single amino acid substitutions in the highly conserved IYTS6TGTGXPK motif (amino acids 247–257) impair the function of FATP1 in fatty acid transport without altering its ability to be expressed or trafficked to the plasma membrane. This motif may play a role in binding ATP or in generation of an adenylated intermediate. Consistent with this model, our previous studies indicated that mutation of serine 250 to alanine or threonine 252 to alanine within this motif results in an FATP1 molecule that cannot be efficiently cross-linked to azido-ATP, unlike the wild type FATP1 that is readily cross-linked. We extend these observations in the present study to show that FATP1 molecules containing the serine 250 to alanine mutation have dominant inhibitory activity in fatty acid uptake assays.

We did not observe a decrease in fatty acid uptake in NIH 3T3 or COS7 cells transfected with the non-functional FATP1s250a mutant alone compared with untransfected cells. Both NIH 3T3 and COS7 cells have minimal endogenous FATP1 expression (present study and Refs. 2, 9, and 14). Although it is not possible to rule out a role for low level FATP1 expression in the low level of basal fatty acid uptake in these cells, this uptake may be mediated by other proteins in these cells, such as CD36 (21) or FABPpm (22). On the other hand, this uptake may be non-protein-mediated (i.e. flip-flop) (23, 24). Expression of the FATP1s250a mutant would not be expected to affect fatty acid uptake mediated by these other mechanisms. Thus, to show dominant inhibitory function of the FATP1s250a mutant, we first expressed wild type FATP1 in these cells and then examined the effects of increasing amounts of the FATP1s250a mutant. We observed a clear, dosage-dependent effect, with greater decreases in fatty acid uptake as the ratio of mutant/wild type FATP1 increases. We have also observed that NIH 3T3 cells co-transduced with mutant and wild type FATP1 retroviruses have decreased fatty acid uptake compared with NIH 3T3 cells transduced with wild type FATP1 virus alone (not shown).

To identify the minimal region necessary for dimerization, we employed a series of FATP1 truncation constructs. Although the carboxyl-terminal domain (amino acids 476–646) does not associate with residues 191–475 and does not self-associate, the region containing residues 191–475 is capable of self-association. This suggests that this region may be sufficient for dimerization of full-length FATP1 molecules. Although residues 191–257 are hydrophilic, hydrophobic interactions involving residues between amino acids 258 and 475 may contribute to oligomerization. Unfortunately, we were unable to assess the contribution of the hydrophobic amino-terminal region of FATP1 to dimerization, because truncation constructs containing amino acids 1–190 could not be expressed. Although truncation constructs enable assessment of the contribution of various structural elements to dimerization, these constructs are unlikely to support the fatty acid transport function of FATP1 because they lack significant regions of FATP1 sequence. In future studies, more detailed site-directed mutagenesis may facilitate analysis of the potential contributions to dimerization of specific residues in both the amino-terminal and middle section of FATP1. Such constructs may also be useful in assessing whether dimerization plays a role in the synthesis and targeting of FATP1 to the plasma membrane and in its stability there.

The observation that FATP1 present in Triton X-100 lysates from different cells are unable to associate in vitro suggests that dimerization requires the structures and/or proteins present normally in cells expressing FATP1. Formation of FATP1 dimers may require the membrane-associated conformation of FATP1. On the other hand, FATP1 dimerization may depend on the presence of chaperones in the endoplasmic reticulum or Golgi. FATP1 is co-translationally inserted into membranes and likely traffics from the endoplasmic reticulum to the Golgi to intracellular vesicles (25) prior to its appearance at the plasma membrane. It is unclear at what point FATP1 dimerization occurs. Formally, it is possible that FATP1 dimerizes due to indirect interactions, with another cellular protein at the interface of two FATP1 molecules, although the 130-kDa oli-
gomer is most consistent with an FATP1 dimer. The observation that FATP1 molecules co-immunoprecipitate in a range of detergent solubilization conditions suggest that dimerization is not a detergent-specific effect.

Although our studies provide evidence for FATP1 dimers as constituents of the cell surface fatty acid transport complex, it is possible that these FATP1 molecules associate with other, as yet to be determined molecules that might play a role in facilitating or regulating this important physiologic process. Both our Western blot studies of non-boiled samples and our sucrose gradient analysis suggest that higher molecular weight complexes exist around 200 kDa. These could represent higher order oligomers of FATP1 or association of FATP1 dimers with one or more other proteins. Future studies in our laboratory will be aimed at identifying the molecular composition of these complexes.

In summary, we have demonstrated for the first time that FATP1 is capable of dimerization. Moreover, dimerization of FATP1 molecules plays a critical role in fatty acid transport across the plasma membrane of mammalian cells. The dominant negative activity identified in FATP mutant s250a will be useful in probing the role of FATP-mediated fatty acid transport in both cultured cells and in animal models. Finally, the ability of FATP1 to form oligomers provides additional clues regarding FATP1 function that will guide future structure-function studies.

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